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31 January–2 February 2017

SYMPOSIUM CHAIRS:

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10037-1, Session 1

Handheld spatial frequency domain spectrographic imager for depth-sensitive, quantitative spectroscopy of skin tissue

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Spatial Frequency Domain Spectroscopy (SFDS) is an optical technique that quantitatively characterizes structured tissue composition in depth; a critical need for both non-invasive Melanoma screening and staging. The development of this technique and associated depth sensitive models, however, have been based off of a benchtop, point-spectroscopy system that is cumbersome to use and transport, thereby limiting its translation to investigations involving clinical populations. To that end, a handheld, line imaging implementation of SFDS has been fabricated.

We present the design of this portable system that not only enables SFDS to be used in clinical settings, but also generates images of tissue in depth. This instrument features a modified commercial micro-projector to deliver custom, broadband illumination patterns on to tissue and a custom, compact line imaging spectrometer to collect diffusely reflected visible and near infrared light at ~1nm spectral resolution and ~50 micron spatial resolution at the surface of tissue. The system performance was validated through phantom studies, using the original SFDS point-spectroscopy system as reference. Initial in-vivo results from pigmented lesions acquired under IRB approved protocols are also provided to illustrate the potential for this model-based, depth segmentation imaging modality.

10037-2, Session 1

Spectral biopsy for skin cancer diagnosis: initial clinical results

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Skin cancer is the most common form of cancer in the United States and is a recognized public health issue. Diagnosis of skin cancer involves biopsy of the suspicious lesion followed by histopathology. Biopsies, which involve excision of the lesion, are invasive, at times unnecessary, and are costly procedures (~$2.8B/year in the US). An unmet critical need exists to develop a non-invasive and inexpensive screening method that can eliminate the need for unnecessary biopsies. To address this need, our group has reported on the continued development of a noninvasive method that utilizes multimodal spectroscopy towards the goal of a “spectral biopsy” of skin. Our approach combines Raman spectroscopy, fluorescence spectroscopy, and diffuse reflectance spectroscopy to collect comprehensive optical property information from suspicious skin lesions. We previously described an updated spectral biopsy system that allows acquisition of all three forms of spectroscopy through a single fiber optic probe and is composed of off-the-shelf OEM components that are smaller, cheaper, and enable a more clinic-friendly system. We present initial patient data acquired with the spectral biopsy system, the first from an extensive clinical study (n = 250) to characterize its performance in identifying skin cancers (basal cell carcinoma, squamous cell carcinoma, and melanoma). We also present our first attempts at analyzing this initial set of clinical data using statistical-based models, and with models currently being developed to extract biophysical information from the collected spectra, all towards the goal of noninvasive skin cancer diagnosis.

10037-3, Session 1

Intraoperative imaging of nonmelanoma skin cancers using polarization-enhanced reflectance and fluorescence technique

Anna N. Yaroslavsky, Xin Feng, Univ. of Massachusetts Lowell (United States); Victor A. Neel, Massachusetts General Hospital (United States)

Nonmelanoma skin cancer (NMSC) is the most common human cancer. It is often curable by surgery. Therefore, there is a strong need for accurate removal of these neoplasms, to ensure higher cure rate combined with maximum tissue preservation. Polarization-enhanced reflectance and fluorescence imaging (PERFI) has been reported as a new bedside method that uses fluorescent chromophores to image NMSC ex vivo. This study extends the use of PERFI to in-vivo intraoperative imaging of NMSC.

In this pilot study subjects were recruited from patients with biopsy-confirmed nonmelanoma skin cancer, scheduled to be treated by Mohs micrographic surgery. Sterile methylene blue (MB) was diluted to 0.2 mg/cc in anaesthetic solution and infused by deep dermal infiltration into the peritumoral space. After in vivo reflectance and fluorescence imaging, Mohs surgery was performed. After the first stage of Mohs, the surgical wound was re-imaged. Each excised piece of skin was imaged ex vivo. Then the excised lesion was processed for routine histopathology. Optical images were processed and compared with histopathology. Eight cases were imaged. In all subjects, the contrast agent, MB, was preferentially retained in the tumor. The injection of MB was well tolerated. We observed a transient blue staining of the treated area, which disappeared completely within 1 week in all of the patients. The ex vivo images correlated well with histopathology. In vivo images qualitatively delineated the tumor margins. Based on these results we concluded that the developed technique may provide an efficient rapid intraoperative optical tool for demarcating NMSC during surgery.

10037-4, Session 1

Skin mircorelief as a diagnostic tool

Lioudmila Tchvialeva, Jamie Phillips, Haishan Zeng, BC Cancer Research Ctr. (Canada); David McLean, Harvey Lui, The Univ. of British Columbia (Canada); Tim K. Lee, BC Cancer Research Ctr. (Canada)

Skin surface roughness is an important property for differentiating skin diseases. Recently, roughness has also been identified as a potential diagnostic indicator in the early detection of skin cancer. Objective quantification is usually carried out by creating silicone replicas of the skin and then measuring the replicas. We have developed an alternative in-vivo technique to measure skin roughness based on laser speckle. Laser speckle is the interference pattern produced when coherent light is used to illuminate a rough surface and the backscattered light is imaged. Acquiring speckle contrast measurements from skin phantoms with controllable roughness, we created a calibration curve by linearly interpolating between measured points. This calibration curve accounts for internal scattering and is designed to evaluate skin microrelief whose root-mean-square roughness is in the range of 10-60 micrometers. To validate the effectiveness of our technique, we conducted a study to measure 243 skin lesions including actinic keratosis (8), basal cell carcinoma (24), malignant melanoma (31), nevus (73), squamous cell carcinoma (19), and seborrheic keratosis (79). The average roughness values ranged from 26 to 57 micrometers. Malignant melanoma was ranked as the smoothest and squamous cell carcinoma as the roughest lesion. An ANOVA test confirmed that malignant melanoma has significantly smaller roughness than other lesion types. Our results suggest that skin microrelief can be used to detect malignant melanoma from other skin conditions.

10037-5, Session 1

Physiological basis for noninvasive skin cancer diagnosis using diffuse reflectance spectroscopy

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Diffuse reflectance spectroscopy offers a noninvasive, fast, and low-cost alternative to visual screening and biopsy for skin cancer diagnosis. We have previously acquired reflectance spectra from 137 lesions in 76 patients and determined the capability of spectral diagnosis using principal component analysis (PCA). However, it is not well elucidated why spectral analysis enables tissue classifications. To provide the physiological basis, we used the Monte Carlo look-up table (MCLUT) model to extract physiological parameters from those clinical data. The MCLUT model results in the following physiological parameters: oxygen saturation, hemoglobin concentration, melanin concentration, vessel radius, and scattering parameters. Based on these physiological parameters, logistic regression classifiers were created, and classification results were compared with histopathology of the lesions. Using numerical cut-offs for these physiological parameters, we achieved higher sensitivity and specificity for most of classifications compared to our previous PCA results. The best classification is basal cell carcinomas versus normal skin with a sensitivity and specificity of 100% and 84%, respectively. Physiological parameters show that cancerous skin tissue has significantly lower oxygen saturation, higher hemoglobin concentration, lower scattering, and larger vessel radius, compared to normal tissue. These results demonstrate the potential of diffuse reflectance spectroscopy for detection of early precancerous changes in tissue. In addition, a diagnostic algorithm that combines these physiological parameters holds promise for a non-invasive diagnosis of skin cancer. Our model provides insight to cancer physiology by extracting physiological parameters that pathologists are familiar with.

10037-39, Session 1

Quantification of changes in skin hydration and sebum after tape stripping using Infrared spectroscopy

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The stratum corneum is the outermost layer of the epidermis and it plays the role of the barrier to water loss. Stratum corneum is composed of the corneocytes and an intercellular lipid bilayer matrix. The hydration and sebum retaining ability of the skin is primarily related to the stratum corneum [1]. Optimal balance between skin lipids and water is reported as essential indicator of skin integrity and functionality, whereas disrupted balance is found in different dermatological disorders such as psoriasis, atopic eczema, edema, rosacea [2]. Tape stripping of human stratum corneum has been used in skin physiology research for example to measure skin barrier function, to quantify the penetration of drugs and to evaluate different skin disorders [3].

Many biophysical methods have been reported for measuring skin hydration and sebum levels independently. However, no non-contact devices and methods have been reported for the quantitative spatial mapping of these components simultaneously. Recently we demonstrated the feasibility of a non-invasive short wave infrared spectroscopic technique for simultaneous measurement of oiliness and hydration levels of the skin [4]. The method is based on differential detection in the spectral region around 1720 nm between the optimal wavelengths corresponding to the lipid vibrational bands that lay “in between” the prominent water absorption bands.

The aim of this study is to quantify the depth resolved changes in skin hydration and sebum levels after tape stripping using the infrared spectroscopic set-up and compare the results with conventional devices such as Corneometer and Sebumeter. We demonstrate that differential detection in the spectral range around 1720 nm allows accurate and sensitive depth profiling of stratum corneum sebum and hydration levels. We anticipate that short wave infrared spectroscopic technique combined with tape stripping can provide much more-quantitative and more reliable skin barrier function information in contrast to conventionally employed biophysical methods.

10037-6, Session 2

Wavenumber selection based analysis in Raman spectroscopy improves skin cancer diagnostic specificity at high sensitivity levels

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Background: Raman spectroscopy is a non-invasive optical technique which can measure molecular vibrational modes within tissue. A large-scale clinical study (n = 518) has demonstrated that real-time Raman spectroscopy could distinguish malignant from benign skin lesions with good diagnostic accuracy; this was validated by a follow-up independent study (n = 127). Objective: Most of the previous diagnostic algorithms have typically been based on analyzing the full band of the Raman spectra, either in the fingerprint or high wavenumber regions. Our objective in this presentation is to explore wavenumber selection based analysis in Raman spectroscopy for skin cancer diagnosis. Methods: A wavenumber selection algorithm was implemented using variably-sized wavenumber windows, which were determined by the correlation coefficient between wavenumbers. Wavenumber windows were chosen based on accumulated frequency from leave-one-out cross-validated stepwise regression or least and shrinkage selection operator (LASSO). The diagnostic algorithms were then generated from the selected wavenumber windows using multivariate statistical analyses, including principal component and general discriminant analysis (PC-GDA) and partial least squares (PLS). A total cohort of 645 confirmed lesions from 573 patients encompassing skin cancers, precancers and benign skin lesions were included. Lesion measurements were divided into training cohort (n = 518) and testing cohort (n = 127) according to the measurement time. Result: The area under the receiver operating characteristic curve (ROC) improved from 0.861–0.891 to 0.891–0.911 and the diagnostic specificity for sensitivity levels of 0.99-0.90 increased respectively from 0.17–0.65 to 0.20–0.75 by selecting specific wavenumber windows for analysis. Conclusion: Wavenumber selection based analysis in Raman spectroscopy improves skin cancer diagnostic specificity at high sensitivity levels.

10037-7, Session 2

Melanoma and basal cell carcinoma control with Raman and fluorescence spectroscopy in visible and NIR regions

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One of the most dangerous forms of cancer is malignant melanoma as

melanomas cause more than 76% of skin cancer deaths. Russia cancer mortality rates are nearly twice as high as in the UK or the USA. In this regard it is necessary to find a new instrumental ways of early cancer detection. The aim of this study was to develop a rapid, highly sensitive method of tumor analysis involving the combination of RS and AF techniques in visible and NIR regions.

All experimental studies were approved by the ethical committee of Samara State Medical University. AF was stimulated in visible and NIR regions by two lasers 457nm and 785 nm, while Raman signal was acquired only in NIR region. More than 100 skin tissue samples containing melanoma and basal cell carcinoma were tested. All spectra were registered for neoplasms and surrounding normal tissues. Shape of AF and Raman spectra is caused by porphyrins, keratins, flavins, melanin and lipids. For tissue type determination six criteria were used. These criterions uses intensity of Raman bands in 1340, 1450 and 1650 cm-1, AF spectral local maxima positions and intensity, and AF curvature in NIR region.

For melanoma and basal cell carcinoma separation every criterion demonstrates accuracy from 58 to 83%. Combined applying of these criteria allows for skin cancer detection with more than 96% accuracy. These results demonstrate high potential of the proposed method, as analysis of Raman and AF spectra is simple and may be used in mass screening applications.

10037-9, Session 3

Skin cancer margin analysis within minutes with FFOCT

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Non-melanoma skin cancer (NMSC) is the most common cancer. Treatment consists of surgical removal of the skin cancer. Traditional excision involves the removal of the visible skin cancer with a significant margin of normal skin. On cosmetically sensitive areas, Mohs micrographic tissue is the standard of care. Mohs uses intraoperative microscopic margin assessment which minimizes the surgical defect and can help reduce the recurrence rate by a factor of 3. The current Mohs technique relies on frozen section tissue slide preparation which significantly lengthens operative time and requires on-site trained histotechnicians. Full-Field Optical Coherence Tomography (FFOCT) is a novel optical imaging technique which provides a quick and efficient method to visualize cancerous areas in minutes, without any preparation or destruction of the tissue. This study aimed to evaluate the potential of FFOCT for the analysis of skin cancer margins during Mohs surgery.

Over 150 images of Mohs specimens were acquired intraoperatively with FFOCT before frozen section analysis. The imaging procedure took less than 5 minutes for each specimen. No artifacts on histological preparation were found arising from FFOCT manipulation; however frozen section artifact was readily seen on FFOCT. An atlas was established with FFOCT images and corresponding histological slides to reveal FFOCT reading criteria of normal and cancerous structures. Blind analysis showed high concordance between FFOCT and histology.

FFOCT can potentially reduce recurrence rates while maintaining short surgery times, optimize clinical workflow, and decrease healthcare costs. For the patient, this translates into smaller infection risk, decreased stress, and better comfort.

10037-10, Session 3

Peri-operative imaging of cancer margins with reflectance confocal microscopy during Mohs micrographic surgery: feasibility of a videomosaicing algorithm

Eileen S. Flores, Oriol Yelamos, Miguel A. Cordova, Kivanc Kose, William Phillips, Anthony Rossi, Kishwer Nehal, Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Reflectance confocal microscopy (RCM) imaging shows promise for guiding surgical treatment of skin cancers. Recent technological advancements such as the introduction of the handheld version of the reflectance confocal microscope, video acquisition and video-mosaicking have improved RCM as an emerging tool to evaluate cancer margins during routine surgical skin procedures such as Mohs micrographic surgery (MMS). Detection of residual non-melanoma skin cancer (NMSC) tumor during MMS is feasible, as demonstrated by the introduction of real-time perioperative imaging on patients in the surgical setting. Our study is currently testing the feasibility of a new mosaicking algorithm for peri-operative RCM imaging of NMSC cancer margins on patients during MMS.

We report progress toward imaging and image analysis on sixty patients, who presented for MMS at the MSKCC Dermatology service. The first 10 patients were used as a training set to establish an RCM imaging algorithm, which is being implemented on the remaining test set of 50 patients. RCM imaging, using 35% AlCl3 for nuclear contrast, was performed pre- and intra-operatively with the Vivascope 3000 (Caliber ID). Imaging was performed in quadrants in the wound, to simulate the Mohs surgeon's examination of pathology. Videos were taken at the epidermal and deep dermal margins. Our Mohs surgeons assessed all videos and video-mosaics for quality and correlation to histology.

Overall, our RCM video-mosaicking algorithm is feasible. RCM videos and video-mosaics of the epidermal and dermal margins were found to be of clinically acceptable quality. Assessment of cancer margins was affected by type of NMSC, size and location. Among the test set of 50 patients, 10 cases have been analyzed thus far and these show acceptable imaging quality, resolution and contrast. Visualization of nuclear and cellular morphology of residual BCC tumor and normal skin features could be detected in the peripheral and deep dermal margins. We observed correlation between the RCM videos/video-mosaics and the corresponding histology for presence of tumor in all 10 lesions. Further analyses of the remaining 40 cases are in progress.

Peri-operative RCM imaging shows promise for improved and faster detection of cancer margins and guiding MMS in the surgical setting.

10037-11, Session 3

Deep learning based classification of morphological patterns in reflectance confocal microscopy to guide noninvasive diagnosis of melanocytic lesions

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In this study we present a deep learning based classification algorithm

for discriminating morphological patterns that appear in RCM mosaics of melanocytic lesions collected at the dermal epidermal junction (DEJ). These patterns are classified into 6 distinct types in the literature: background, meshwork, ring, clod, mixed, and aspecific. Clinicians typically identify these morphological patterns by examination of their textural appearance at 10X magnification. To mimic this process we divided mosaics into smaller regions, which we call tiles, and classify each tile in a deep learning framework. We used previously acquired DEJ mosaics of lesions deemed clinically suspicious, from 20 different patients, which were then labelled according to those 6 types by 2 expert users. We tried three different approaches for classification, all starting with a publicly available convolutional neural network (CNN) trained on natural image, consisting of a series of convolutional layers followed by a series of fully connected layers: (1) We fine-tuned this network using training data from the dataset. (2) Instead, we added an additional fully connected layer before the output layer network and then re-trained only last two layers, (3) We used only the CNN convolutional layers as a feature extractor, encoded the features using a bag of words model, and trained a support vector machine (SVM) classifier. Sensitivity and specificity were generally comparable across the three methods, and in the same ranges as our previous work using SURF features with SVM . Approach (3) was less computationally intensive to train but more sensitive to unbalanced representation of the 6 classes in the training data. However we expect CNN performance to improve as we add more training data because both the features and the classifier are learned jointly from the data.

*First two authors share first authorship.

10037-12, Session 3

Video-mosaicking of in vivo reflectance confocal microscopy images for noninvasive examination of skin lesions

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In this report we describe a computer vision based pipeline to convert in-vivo reflectance confocal microscopy (RCM) videos collected with a handheld system into large field of view (FOV) mosaics. For many applications such as imaging of hard to access lesions, intraoperative assessment of MOHS margins, or delineation of lesion margins beyond clinical borders, raster scan based mosaicing techniques have clinically significant limitations. In such cases, clinicians often capture RCM videos by freely moving a handheld microscope over the area of interest, but the resulting videos lose large-scale spatial relationships. Videomosaicking is a standard computational imaging technique to register, and stitch together consecutive frames of videos into large FOV high resolution mosaics. However, mosaicing RCM videos collected in-vivo has unique challenges: (i) tissue may deform or warp due to physical contact with the microscope objective lens, (ii) discontinuities or “jumps” between consecutive images and motion blur artifacts may occur, due to manual operation of the microscope, and (iii) optical sectioning and resolution may vary between consecutive images due to scattering and aberrations induced by changes in imaging depth and tissue morphology. We addressed these challenges by adapting or developing new algorithmic methods for videomosaicking, specifically by modeling non-rigid deformations, followed by automatically detecting discontinuities (cut locations) and, finally, applying a data-driven image stitching approach that fully preserves resolution and tissue morphologic detail without imposing arbitrary pre-defined boundaries. We will present example mosaics obtained by clinical imaging of both melanoma and non-melanoma skin cancers. The ability to combine freehand mosaicing for handheld microscopes with preserved cellular resolution will have high impact application in diverse clinical settings, including low-resource healthcare systems.

10037-13, Session 4

In vivo multiphoton microscopy of the eyelid skin

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Multiphoton microscopy (MPM) has become an important imaging method for non-invasive and high-resolution imaging of the skin. Due to the nonlinear excitation three dimensional information is intrinsically provided. In combination with fluorescence lifetime imaging microscopy, it is possible to obtain both structural and metabolic data.

Human in vivo measurements are usually limited to easy accessible regions. However, often specific body part such as the eyelid are of interest for the cosmetic industry. By using the multiphoton certified clinical imaging tomograph MPTflex this limitation can be overcome. It’s articulated mirror-arm and scanning head enables the measurement of otherwise difficult access areas.

We were able to characterize the epidermal and dermal layers of the eyelid skin of human volunteers in vivo using the endogenous autofluorescence intensity, lifetime, and second-harmonic generation signals. Skin properties such epidermal and epidermal-dermal junction thicknesses were also assessed. The influence of eye cosmetic products on the skin was investigated.

10037-14, Session 4

In vivo multiphoton-microscopy of laser-induced optical breakdown in human skin

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We use a multiphoton microscopy (MPM)-based clinical microscope (MPTflex, JenLab, Germany) to describe changes in human skin following treatment with a fractional non-ablative laser (PicoWay, Candela).

The treatment was based on a fractionated picosecond Nd:YAG laser (1064 and 532nm, 3mJ and 1.5mJ (no attenuation), respectively maximum energy/pulse, 100 microbeams/6mmx6mm). Improvements in skin appearance resulting from treatment with this laser have been noted but optimizing the efficacy depends on a thorough understanding of the specific skin response to treatment.

MPM is a nonlinear laser scanning microscopy technique that features sub-cellular resolution and label-free molecular contrast. MPM contrast in skin is derived from second-harmonic generation of collagen and two-photon excited fluorescence of NADH/FAD+, elastin, keratin, melanin.

In this pilot study, two areas on the arm of a volunteer (skin type II) were treated with the picoWay laser (1064nm, 3mJ; 532nm, 1.5mJ; 1pass). The skin response to treatment was imaged in-vivo at 8 time points over the following 4 weeks. MPM revealed micro-injuries present in epidermis. Damaged individual cells were distinguished after 3h and 24h from treatment with both wavelengths. Pigmented cells were particularly damaged in the process, suggesting that melanin is the main absorber and the primary target for laser induced optical breakdown. At later time points, clusters of cellular necrotic debris were imaged across the treated epidermis. These results represent the groundwork for future longitudinal

studies on expanded number of subjects to understand the response to treatment in different skin types at different laser parameters, critical factors in optimizing treatment outcomes.

10037-15, Session 4

3D imaging of hematoxylin and eosin stained thick tissues with a sub-femtoliter resolution by using Cr:forsterite-laser-based nonlinear microscopy

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Intraoperative assessment of excision tissues during cancer surgery is clinically important. The assessment is used to be guided by the examination for residual tumor with frozen pathology, while it is time consuming for preparation and is with low accuracy for diagnosis. Recently, reflection confocal microscopy (RCM) and nonlinear microscopy (NLM) were demonstrated to be promising methods for surgical border assessment. Intraoperative RCM imaging may enable detection of residual tumor directly on skin cancers patients during Mohs surgery. The assessment of benign and malignant breast pathologies in fresh surgical specimens was demonstrated by NLM. Without using hematoxylin and eosin (H&E) that are common dyes for histopathological diagnosis, RCM was proposed to image in vivo by using aluminum chloride for nuclear contrast on surgical wounds directly, while NLM was proposed to detect two photon fluorescence nuclear contrast from acrdine orange staining. In this paper, we propose and demonstrate 3D imaging of H&E stained thick tissues with a sub-femtoliter resolution by using Cr:forsterite-laser-based NLM. With a 1260 nm femtosecond Cr:forsterite laser as the excitation source, the hematoxylin will strongly enhance the third-harmonic generation (THG) signals, while eosin will illuminate strong fluorescence under three photon absorption. Compared with previous works, the 1260 nm excitation light provide high penetration and low photodamage to the exercised tissues so that the possibility to perform other follow-up examination will be preserved. The THG and three-photon process provides high nonlinearity so that the super resolution in 3D is now possible. The staining and the contrast of the imaging is also fully compatible with the current clinical standard on frozen pathology thus facilitate the rapid intraoperative assessment of excision tissues. This work is sponsored by National Health Research Institutes and supported by National Taiwan University Hospital.

10037-16, Session 5

In vivo characterization of structural and optical properties of human skin by combined photothermal radiometry and diffuse reflectance spectroscopy

Nina Verdel, Jo?ef Stefan Institute (Slovenia); Ana Marin, Faculty of Mathematics and Physics, Univ. of Ljubljana (Slovenia); Luka Vidovi?, Jo?ef Stefan Institute (Slovenia); Matija Milani?, Jo?ef Stefan Institute (Slovenia) and Faculty of Mathematics and Physics, Univ. of Ljubljana (Slovenia); Boris Majaron, Jo?ef Stefan Institute (Slovenia)

We have recently demonstrated the potential of pulsed photothermal radiometry (PPTR) for in vivo characterization of laser interaction with human skin in several relevant scenarios, both in research and clinical settings. Most of these applications involved reconstruction of the laser-induced temperature depth profiles by solving the inverse problem of heat diffusion and infrared emission form the skin surface.

Quantitative assessment of the structural properties and presence of specific chromophores (e.g., melanin, hemoglobin) at the irradiated site, however, requires solving an additional inverse problem, namely that of light transport inside the skin. This process hinges on several assumptions, such as the scattering properties of the involved tissues. While the latter are listed in literature, they are also lkely to vary with anatomical location, person's age, gender, lifestyle (smoking), etc., which inevitably introduces systematic errors to the assessed values.

In an attempt to resolve this issue, we complement the PPTR measurements with another non-invasive technique, diffuse reflectance spectroscopy (DRS) in the visible spectral range. Both data sets are analyzed using numerical models of optical and thermal transport in multi-layered tissue structures, coupled with multidimensional optimization algorithms (i.e., inverse Monte Carlo). This avoids the limited validity of the diffuse approximation solutions near the irradiated skin surface and enables us to account for the finite diameter of the sample opening on the integrating sphere used for DRS measurements.

We find that combining the two techniques considerably improves the accuracy and robustness of structural and spectroscopic characterization of human skin at the selected test site.

10037-18, Session 5

Quantitative assessment of graded burn wounds using a commercial and research grade laser speckle imaging (LSI) system

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Burn wounds are often characterized by injury depth which then dictates wound management strategy. While most superficial burns and full thickness burns can be diagnosed through visual inspection, clinicians have difficulty accurately diagnosing burns that fall between these extremes. Accurately diagnosing burn severity in a timely manner is critical for starting appropriate treatment plans at the earliest time points to improve patient outcomes. To address this challenge, research groups have studied the use of commercial laser Doppler imaging (LDI) systems to objectively characterize burn-wound severity. Despite initial promising findings, LDI systems are not commonplace in part due to the limited utility of LDI during the initial 48 hours after burn injury. Such a delay decreases the efficacy of LDI and inevitably contributes to an increased length of stay for burn patients. Furthermore, commercial LDI systems are being phased out in favor of laser speckle imaging (LSI) systems that can provide similar information with faster acquisition speeds. Here we studied the performance of a commercial LSI system (Pericam PSI, Perimed AB) in a controlled environment using intralipid (1%) flowing through a tissue-simulating phantom, and also in a controlled burn study in which wounds of graded severity were created on a Yorkshire pig. The burn wounds were monitored for five days and burn depths verified using histological analysis. In addition to the commercial LSI system, a research grade LSI system was used to compare quantitatively the performance of both systems and also better understand the "Perfusion Unit" output of commercial systems.

10037-19, Session 5

Findings toward the miniaturization of a laser speckle contrast device for skin roughness measurements

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Skin roughness is an important parameter in the characterization of skin and skin lesions, particularly for the purposes of skin cancer detection. Our group had previously constructed a laser speckle contrast device that is able to detect the roughness in microrelief of the skin. This paper reports on findings made for the further miniaturization of our existing portably-sized device. These findings include the feasibility of adopting a laser diode without temperature control, and the use of a single CCD camera for detection. The coherence length of a laser is a crucial criterion for speckle measurements as it must be within a specific range. The coherence length of a commercial grade 405 nm laser diode was found to be of an appropriate length. Also, after a short warm-up period the coherence length of the laser was found to remain relatively stable, even without temperature control. Although the laser's temperature change during operation may affect its power output and the shape of its spectrum, these are only minor factors in speckle contrast measurements. Our second finding is the construction of a calibration curve to relate speckle measurements to roughness using only parallel polarization from one CCD camera. This was created using experimental data from metal roughness standards, and validated using measurements on skin phantoms and in-vivo skin. These improvements are important steps forward in the ongoing development of the laser speckle contrast device, especially towards a clinical device to measure skin roughness and evaluate skin lesions.

10037-20, Session 6

Optical microscopy of targeted drug delivery and local distribution in skin of a topical minocycline: implications in translational research and guidance for therapeutic dose selection

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Acne vulgaris is a chronic inflammatory skin condition commonly resulting in negative aesthetic and social impacts on those affected. Minocycline, currently available as an oral antibiotic for moderate to severe acne, has a known minimum inhibitory concentration (MIC) for the acne-causing bacterium Propionibacterium acnes (P. acnes) in vitro, with its anti-inflammatory properties also eliciting inhibitory effects on pro-inflammatory molecules. A novel topical gel composition containing solubilized minocycline (BPX-01) has been developed to directly deliver the drug to the skin. Because minocycline is a known fluorophore, fluorescence microscopy and concurrent quantitative measurements were performed on excised human facial skin dosed with different concentrations, in order to determine the spatial distribution of the drug and quantification of its local concentration in the epidermis and the pilosebaceous unit where P. acnes generally reside. Local minocycline delivery confirmed achievement of an adequate therapeutic dose to support clinical studies. Subsequently, a 4-week double-blind, randomized, vehicle controlled clinical study was performed to assess the safety and efficacy of 1% minocycline BPX-01 applied daily. No instances of cutaneous toxicity were reported, and a greater than 1 log reduction of P. acnes count was observed at week 4 with statistical significance from baseline and vehicle control. In addition, no detectable amounts of minocycline in the plasma were reported, suggesting the potential of this new formulation to diminish the known systemic adverse effects associated with oral minocycline. Follow-on clinical plans are underway to further establish the safety of BPX-01 and to evaluate its efficacy against inflammatory acne lesions in a 225 patient multi-center dose-finding study.

10037-21, Session 6

Light emitting fabric for photodynamic treatment of actinic keratosis

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Induced by sun damages, actinic keratosis (AK) are usually present on the scalp, shoulders or arms of the patients and thus are easily reachable by light, making photodynamic therapy (PDT) one of the first line treatments. The planar shape of current light sources used for PDT does not permit to deliver a homogeneous light on lesions located on curved parts of the human body, and may leads to under-treatment of some of these lesions. Moreover, PDT is known to be very painful, and may force the patient to ask for premature end of the treatment.

To address these issues, a new device based on light emitting fabrics (LEF) was developed. The integration of optical fibers into flexible textile structures, by using knitting or weaving processes was the first step of our work.

The predetermined macro-bending of optical fibers, led to a homogeneous side emission of light over the entire surface of the fabric. Tests showed that additional curvatures when applying the LEF on non-planar surfaces had no impact on light delivery and proved that LEF can adapt to the human morphology.

The ability of the LEF, coupled with a 635nm LASER source, to deliver a homogeneous light to lesions is currently assessed in two clinical trials for the treatment of AK of the scalp by PDT. The low irradiance and progressive activation of the photosensitizer ensured a significant pain reduction (0,3 ± 0,6 /10), compared to discomfort levels experienced by patients during a conventional PDT session (4,8 ± 1,3 /10).

10037-22, Session 6

Measuring temperature induced phase change kinetics in subcutaneous fatty tissues using near infrared spectroscopy (NIRS), magnetic resonance spectroscopy and optical coherence tomography

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Monitoring phase transition in adipose tissue and formation of lipid crystals is important in Cryo-procedures such as cryosurgery or Selective Cryolipolysis (SC). In this work, we exploited a Near-Infrared Spectroscopy (NIRS) method to monitor the onset of fat freezing/melting. Concurrent

measurements using frequency domain NIRS and MR Spectroscopy during cooling/heating were performed on an in vitro porcine skin sample with a thick subcutaneous fat layer in a human MR scanner. The NIRS probe was placed on the skin measuring the average optical scattering of the fatty layer. Two fiber optic temperature probes were inserted in the area of the MRS and NIRS measurements. To further investigate the microscopic features of the phase-transition, an identical cooling/heating procedure was replicated on the same fat tissue while being imaged by Optical Coherence Tomography. The temperature relationships of optical scattering, MRS peak characteristics and OCT reflection intensity were analyzed to find signatures related to the onset of phase transition.

The optical scattering in the fatty tissues decreases during the heating and increases by cooling. However, there is an inflexion in the rate of change of the scattering while the phase transition happens in the fatty layer. The methylene fat peaks on the MR Spectrum are also shown to be broadened during the cooling. OCT intensity displays a sharp increase at the transition temperature. The results from multiple samples show two transition points around 5-10 ˚C (cooling) and 15-20 ˚C (heating) through all three methods, demonstrating that adipose tissue phase change can be monitored non-invasively.

10037-23, Session 6

The role of ablative lasers in cutaneous scars: tissue regeneration to restore function *(Invited Paper)*

Nathan Uebelhoer, Naval Medical Ctr. San Diego (United States)

Many laser wavelengths with various power and pulse characteristics have been used in an attempt to improve cutaneous scars. No single configuration has produced such dramatic changes in quality of life as the high energy, low density, sub-millisecond pulsed ablative infrared laser. Hundreds of wounded military service members with burn and traumatic scars that resulted in disabling restriction in range of motion have been treated since 2008. By fractionating the pulse to produce a uniform thermal injury less than 400um wide and to a depth of 3mm into the scar, we have observed dramatic reductions in scar-induced pain, pruritus, and most significantly, improvements in range of motion. The clinical and histologic changes seen in restrictive scars following treatment correlates with a regeneration of tissue that appears and functions more like normal tissue rather than scar. This lecture will describe our experience in the military and the latest research to support our observations.

10037-24, Session 7

Hypericin-mediated selective photomodification of connective tissues

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Controlled modification of connective tissue is important for biomedical applications. Through the use of second harmonic generation imaging, two-photon fluorescence microscopy, and spectrofluorimetry, we found that hypericin, a natural pigment, induces photosensitized destruction of collagen fibers. However, this process but does not affect elastic fibers and lipids. We demonstrated the dynamics and efficiency of collagen photomodification and investigated mechanisms of this processes. Our results suggest that hypericin–mediated photoprocesses in biological tissues may be useful in biomedical applications that require selective modification of connective tissues.

10037-25, Session 7

Non-invasive in vivo characterization of skin wound healing using label-free multiphoton microscopy

Jake D. Jones, Fariah Majid, Hallie Ramser, Kyle P. Quinn, Univ. of Arkansas (United States)

Non-healing ulcerative wounds, such as diabetic foot ulcers, are challenging to diagnose and treat due to their numerous possible etiologies and the variable efficacy of advanced wound care products. Thus, there is a critical need to develop new quantitative biomarkers and diagnostic technologies that are sensitive to wound status in order to guide care. The objective of this study was to evaluate the utility of label-free multiphoton microscopy for characterizing wound healing dynamics in vivo and identifying potential differences in diabetic wounds. We isolated and measured an optical redox ratio of FAD/(NADH+FAD) autofluorescence to provide three-dimensional maps of local cellular metabolism. Using a mouse model of wound healing, in vivo imaging at the wound edge identified a significant decrease in the optical redox ratio of the epidermis ($p \leq 0.0103$) between Days 3 through 14 compared to Day 1. This decrease in redox ratio coincided with a decrease in NADH fluorescence lifetime and thickening of the epithelium, collectively suggesting a sensitivity to keratinocyte hyperproliferation. In contrast to normal wounds, we have found that keratinocytes from diabetic wounds remain in a proliferative state at later time points with a lower redox ratio at the wound edge. Microstructural organization and composition was also measured from second harmonic generation imaging of collagen and revealed differences between diabetic and non-diabetic wounds. Our work demonstrates label-free multiphoton microscopy offers potential to provide non-invasive structural and functional biomarkers associated with different stages of skin wound healing, which may be used to detect delayed healing and guide treatment.

10037-26, Session 7

A portable multi-spectral imaging system to monitor the level of melanin and wrinkle during low level light therapy

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There are a number of commercially available low level light therapy (LLLT) devices in a market, and face whitening or wrinkle reduction is one of targets in LLLT. The facial improvement could be known simply by visual observation of face, but it cannot provide either quantitative data or recognize a subtle change. Clinical diagnostic instruments such as mexameter can provide a quantitative data, but it costs too high for home users. Therefore, we designed a low cost multi-spectral imaging device by adding additional LEDs (470nm, 640nm, white LED, 905nm) to a commercial USB microscope which has two LEDs (395nm, 940nm) as light sources. Among various LLLT skin treatments, we focused on getting melanin and wrinkle information. For melanin index measurements, multi-spectral images of nevus were acquired and melanin index values from color image (conventional method) and from multi-spectral images were compared. The results showed that multi-spectral analysis of melanin index can visualize nevus with a different depth and concentration. A cross section of wrinkle on skin resembles a wedge which can be a source of high frequency components when the skin image is Fourier transformed into a spatial frequency domain map. In that case, the entropy value of the spatial frequency map can represent the frequency distribution which is related with the amount and thickness of wrinkle. Entropy values from multi-spectral images can potentially separate the percentage of thin and shallow wrinkle from thick and deep wrinkle. From the results, we found that this low cost multi-spectral imaging system could be beneficial for home users of LLLT by providing the treatment efficacy in a quantitative way.

10037-27, Session 7

In vivo assessment of wound re-epithelialization by UV fluorescence excitation imaging

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Background and Objectives: We had demonstrated a non-invasive, non-contact, fast and simple but robust fluorescence imaging (u-FEI) method to monitor the healing of skin wounds in vitro. This system can image highly-proliferating cellular processes (295/340 nm excitation/emission wavelengths) to study epithelialization in a cultured wound model. The objective of this work is to evaluate u-FEI for studying the wound re-epithelialization in vivo.

Study Design: Full-thickness wounds were created in the tail of rats and imaged weekly using u-FEI at 295/340nm excitation/emission wavelengths. Histology (H&E staining) and immunohistology (pan-keratin) were used to investigate the correlation between the spatial distribution and intensity of fluorescence and the extent of wound epithelialization. In addition, the expression of the nuclear protein Ki67 was used to confirm the association between the proliferation of keratinocyte cells and the intensity of fluorescence.

Results: Keratinocytes forming neo-epidermis exhibited higher fluorescence intensity than the keratinocytes not involved in re-epithelialization. In full-thickness wounds the fluorescence first appeared at the wound edge where keratinocytes initiated the epithelialization process. Next the extent of fluorescence increased towards the center as the keratinocytes partially covered the wound. Subsequently, the fluorescence decreased at the edges and was present only at the centre as the keratinocytes completely covered the wound at week 4. H&E and immunohistology (pan-keratin, Ki67) show that changes in fluorescence intensity from the 295/340nm band correspond to newly formed epidermis.

Conclusions: u-FEI at 295/340nm allows visualization of proliferating keratinocyte cells during re-epithelialization of wounds in vivo, potentially providing a quantitative, objective and simple method for evaluating wound closure in clinic.

10037-28, Session 8

Imaging cold-induced vasodynamic behaviour in skin using optical coherence tomography for microangiography

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In dermatology the reflexes of vasoconstriction and vasodilation are known as important mechanisms of thermoregulation of the inner body. Imaging the physiology of microvasculature of the skin with high spatial resolution in three dimensions while reacting to changes in temperature is crucial for understanding the complex processes of vasodynamics, which result in constriction and dilation of vessels. However, previous studies using Laser-Doppler flowmetry and -imaging could not provide reliable angiographic images which allow to quantify changes in blood vessel diameter. Here, we report a different approach for angiographic imaging of microvasculature of a anaesthetized rodent model using speckle variance optical coherence tomography (svOCT) during and after localized cooling. Therefore a commercial OCT with a center wavelength of 1.3 ?m and a spatial resolution of 13μm was used in combination with a custom built cooling device to image such reflexes at the mouse ear pinna and dorsal skinfold. Cooling was applied in steps of 2?5? C starting at the baseline temperature of 27° C down to ?10° C.

To our surprise and in contrast to the general opinion in literature, we were able to observe that the majority of vessels with a diameter larger than 20 ?m maintain perfused with a constant diameter when the tissue is cooled from baseline to subzero temperatures. However, vasoconstriction was observed very rarely and only in veins, which led to their occlusion. The results of this experiment lead us to reconsider essential aspects of previous understanding of temperature-induced vasodynamics in cutaneous microvasculature.

10037-29, Session 8

Combined multimodal photoacoustic tomography, optical coherence tomography (OCT) and OCT based angiography system for in vivo imaging of multiple skin disorders in human

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All optical photoacoustic tomography (PAT) using a planar Fabry-Perot interferometer polymer film sensor has been demonstrated for in vivo human palm imaging with an imaging penetration depth of 5 mm. The relatively larger vessels in the superficial plexus and the vessels in the dermal plexus are visible in PAT. However, due to both resolution and sensitivity limits, all optical PAT cannot reveal the smaller vessels such as capillary loops and venules. Melanin absorption also sometimes causes difficulties in PAT to resolve vessels. Optical coherence tomography (OCT) based angiography, on the other hand, has been proven suitable for microvasculature visualization in the first couple millimeters in human. In our work, we combine an all optical PAT system with an OCT system featuring a phase stable akinetic swept source. This multimodal PAT/OCT/OCT-angiography system provides us co-registered human skin vasculature information as well as the structural information of cutaneous. The scanning units of the sub-systems are assembled into one probe, which is then mounted onto a portable rack. The probe and rack design gives six degrees of freedom, allowing the multimodal optical imaging probe to access nearly all regions of human body. Utilizing this probe, we perform imaging on patients with various skin disorders as well as on healthy controls. Fused PAT/OCT-angiography volume shows the complete blood vessel network in human skin, which is further embedded in the morphology provided by OCT. A comparison between the results from the disordered regions and the normal regions demonstrates the clinical translational value of this multimodal optical imaging system in dermatology.

10037-30, Session 8

Wide-field and high-sensitive angiography for clinical dermatology applications by swept-source optical coherence tomography

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The visual inspection is considered as an indispensable procedure to provide useful information for the diagnosis in clinical dermatology. Not only the morphological structure but also the functional circulation in human skin is essential for general health, disease diagnosis and treatment monitoring. Among various imaging techniques, optical coherence tomography (OCT) becomes a popular imaging modality due to its capability of non-contact, non-invasive, 3D visualization of both structure and vasculature in human skin with high resolution and high speed. However, most of current OCT systems suffer from short imaging range, which limited the imaging in a small field of view (FOV) and relatively flat area in human skin. Challenge also remains in the visualization of vascular networks within deep dermis layer of human skin that play an important role for blood supply. Here we demonstrate a high-speed swept-source OCT system based on the technique of MEMS tunable vertical cavity surface emission laser (VCSEL) centered at 1310 nm with 100 kHz A-line rate. The single longitudinal mode operation of the laser and high-speed digitizer in our system provide us up to ~ 4.5 cm imaging range with superior roll-off performance. Using this OCT system, we are able to achieve wide FOV (>100 cm2) imaging and high-sensitive angiography in different parts of human skin. The blood vessels from the superficial layer to deep dermis layer of human skin are clearly identified. The presented works demonstrated a remarkable potential of OCT angiography for clinical use in dermatology.

10037-31, Session 8

Characterizing the microcirculation of atopic dermatitis using angiographic optical coherence tomography

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Background and Aim: The microcirculation within localised skin lesions often presents a unique morphology when compared to that of healthy skin. In the case of inflammatory conditions such as atopic dermatitis (AD), epidermal thickening is likely to influence both the depth and shape of the underlying vessels. Optical coherence tomography (OCT) provides a non-invasive view into the tissue, however structural measures of epidermal thickness are made difficult due to the lack of a delineated dermal-epidermal junction in AD patients. Instead, angiographic extensions to OCT may allow for direct measurement of vascular depth, potentially presenting a more robust method of estimating the degree of epidermal thickening.

Methods and results: To investigate microcirculatory changes within AD patients, volumes of angiographic OCT data were collected from 12 healthy volunteers and compared to that of 12 AD patients (Mean SCORAD severity of 20±9). Test sites included the cubital and popliteal fossa, which are commonly affected by AD. Quantitative parameters such as capillary loop density and vascular depth were derived from each dataset and compared between groups. The capillary loops rise to a consistent depth between healthy and AD patients with large capillary loops being indicative of erythema. The superficial vascular plexus is measurably deeper in AD patients, likely as a result of localised inflammation.

Conclusions: Quantifying subtle changes within vascular morphology and depth may give clinicians an indication of disease progression and aid in evaluating the efficacy of treatments. This research was supported by BBSRC grant: BB/F016840/1, with equipment funded by MRC grant: MR/L012669/1.

10037-32, Session 9

Optical coherence tomography for image-guided dermal filler injection and biomechanical evaluation

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Dermal fillers are a very popular anti-aging treatment with estimated sales in the billions of dollars and millions of procedures performed. As the aging population continues to grow, these figures are only expected to increase. Dermal fillers have various compositions depending on their intended application. Reactions to dermal fillers can be severe, such as ischemic events and filler migration to the eyes. However, these adverse reactions are rare. Nevertheless, the capability to perform image-guided filler injections would minimize the risk of such reactions. In addition, the biomechanical properties of various fillers have been evaluated, but there has been no investigation on the effects of filler on the biomechanical properties of skin. In this work, we utilize optical coherence tomography (OCT) for visualizing dermal filler injections with micrometer-scale spatial resolution. In addition, we utilize noncontact optical coherence elastography (OCE) to quantify the changes in the biomechanical properties of pig skin after the dermal filler injections. OCT was successfully able to visualize the dermal filler injection process, and OCE showed that the viscoelasticity of the pig skin was increased locally at the filler injection sites. OCT may be able to provide real-time image guidance in 3D, and when combined with functional OCT techniques such as optical microangiography, could be used to avoid blood vessels during the injection.

10037-33, Session 9

An integrated skin marking tool for use with optical coherence tomography (OCT)

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Non-invasive imaging is a growing field in the assessment of skin diseases. Optical Coherence Tomography (OCT) has sufficient resolution (~10 ?m) and penetration (2 mm) to image lesions that extend into the dermis, allowing the sub-clinical spread of skin cancers to be identified. Currently, repeated removal and replacement of the imaging probe is required for manual marking of pre-surgical margins with a standard surgical marker pen. This degrades the accuracy of margin delineation that could otherwise be achieved under continuous OCT image-guidance. A device has been developed that allows rapid marking of skin without the need to remove the probe or interrupt imaging. The device has been designed, in the first instance, for the probe of the VivoSight 1500 OCT system (MDL, Orpington, UK) but is adaptable for other modalities. We present data on the functionality and accuracy of this system using OCT to guide the placement of marks on phantom materials and the skin of volunteers corresponding to features imaged at depth. The results indicate that imaging, followed by probe-removal and marking of the skin above deep-lying features with a

hand-held surgical marker pen, introduces a root mean square (RMS) error of around 3.7 mm in mark placement compared with an error of >1 mm (RMS) using continuous image-guidance made possible with the device. The device will better enable the application of OCT in guiding the accurate placement of skin marks indicating regions of sub-clinical spread with the aim of achieving complete excision with optimal tissue sparing.

10037-34, Session 9

Comparison of optical localization techniques for optical coherence tomography of the hand for multi-fraction orthovoltage radiotherapy or photodynamic therapy: white light vs. optical surface imaging

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Non-melanoma skin cancer (NMSC) is considered the most commonly diagnosed cancer in the United States and Canada. Treatment options include radiotherapy, surgical excision, radiotherapy, topical therapies, electrocautery, and cryotherapy. For patients undergoing fractionated orthovoltage radiation therapy or photodynamic therapy (PDT), the lesions are typically delineated by clinical markup prior to treatment without providing any information about the underlying tissue thus increasing the risk of geographic miss.

The development of biomarkers for response in NMSC is imperative considering the current treatment paradigm is based on clinical examination and biopsy confirmation. Therefore, a non-invasive image-based evaluation of skin structure would allow for faster and potentially more comprehensive microscopic evaluation of the treated region at the point of care. To address this, our group is investigating the use of optical coherence tomography (OCT) for pre- and post- treatment evaluation of NMSC lesions during radiation therapy and PDT.

Localization of the OCT probe for follow-up is complex, especially in the context of treatment response where the lesion is not present, precluding accurate delineation of the planning treatment area. Further, comparison to standard white light pre-treatment images is limited by the scale of the OCT probe (6 mm X 6 mm) relative to target region.

In this study we compare the set-up accuracy of a typical OCT probe to detect a theoretical lesion on a patient's hand. White light images, optical surface imaging (OSI) and OCT will be obtained at baseline and used for probe set up on subsequent scans. Set-up error will be quantified using advanced image processing techniques.

10037-35, Session PSun

Fabrication of double-layered optical skin phantom with artificial blood vessels embedded for port wine stain laser treatment

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Port Wine Stain (PWS) is malformations of blood vessel in the head and neck regions affecting 0.3-0.5% of children. At birth, PWS presents pink birthmarks on the skin and becomes darker resulting from increase of hemoglobin contents due to expansion of capillaries. Although laser light treatment removes blood vessels in terms of selective photothermolysis (heating and removal of blood vessels), complete removal of the vessels is still challenging. The aim of the current study was to develop artificial PWS phantoms to mimic vascular diseases and to optimize laser parameters reproducibly to improve clinical outcomes. Double-layered polydimethylsiloxane (PDMS)-based skin phantoms were used to emulate human skin tissue with PWS. Hydrophobic PDMS was initially used as a base material for the double-layered phantoms. Coffee and Nigrosin were selected as absorbing agents for epidermis and dermis, respectively. TiO2 was used as a scattering agent for all the layers. The absorption and reduced scattering coefficients of each layer from the fabricated PWS phantoms were estimated by using an inverse adding-doubling (IAD) method with a single integrating sphere. Artificial blood vessels were embedded in the phantom and selectively removed by using 532-nm laser light. Photoacoustic imaging (PAI) was employed to precisely locate the artificial blood vessels with various concentrations before and after the laser treatment. Optical coherence tomography (OCT) confirmed that the thickness of epidermis and dermis were to be approximately 100~150 μm and 2~3mm. The IAD method demonstrated that the absorption and the reduced scattering coefficients were estimated to be around 0.7 and 3.3 mm-1 for epidermis and 0.05 and 2.7 mm-1 for dermis, respectively. Due to strong light absorption by hemoglobin, the 532 nm wavelength was able to selectively eliminate the blood vessels from the PWS phantoms. PAI demonstrated high contrast images of the artificial blood vessels before and after the laser treatment. The double-layered vessel-embedded phantoms can be a feasible model to consistently examine therapeutic parameters for effective and safe laser treatment on various capillaries disorders and birthmarks.

10037-36, Session PSun

Optical virtual biopsy of melasma for the diagnosis, prognosis and therapeutic decision by using in vivo non-invasive harmonic generation microscopy

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Melasma is a common skin disease on the face of hyperpigmentation disorder of melanin and has been the great concern for mid-aged women for the sake of unsightliness. The prevalence rates of melasma, affecting over 5 million people in the U.S., is up to 40% in some females of Southeast Asian populations, and it has brought a huge demand in the field of aesthetic medicine.

Although there are many therapies available for the treatments of melasma, melasma is often a therapeutically challenging disease because there is a great variance concerning efficacy of depigmenting treatments from person to person. Since the quantity and distribution of melanin are the main factors of the therapeutic decision and outcome of melasma, dermatologists desire the information of melanin in skin in order to better assess the responses of treatments and to finally eke out their cure.

In this study, thirty Asian women, between 30 to 65 years old and having symmetrically distributed melasma on the cheeks, were recruited, and we propose a method using harmonic generation microscopy to long-term track the distribution of melanin, melanocytes and melanophages for the therapy of melasma and to help the dermatologists to improve their strategies for curing melasma.

10037-37, Session PSun

Assessment of cylindrical diffusing applicator for 1470 nm Laser lipolysis

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Laser liposuction is a process to liquefy fatty tissue in order to reduce body fat. In order to cylindrically irradiate laser light and to achieve low uniform temperature, a diffusing applicator was designed and developed for thermal treatment. The aim of the current study was to investigate the performance of the cylindrically diffusing applicator for 1470-nm laser lipolysis. Porcine adipose tissue was used as an ex vivo sample for laser lipolysis to emulate human adipose tissue. The cylindrically diffusing applicators were customarily fabricated, and a goniometric system was used to evaluate spatial distribution of the laser light from the applicator. A 1470 nm laser system was employed to perform laser lipolysis due to strong light absorption. Various laser powers and cannula movement speeds were evaluated in terms of weight variation and temperature development to investigate thermal responses of the tissue. The goniometric measurements demonstrated circumferential light distribution form the diffusing tip. The dynamic fiber conditions showed lower thermal gradient than the static condition. The peak temperature of the static fiber condition reached over 150 degree while the dynamic condition generated up to 80 degree at 5 cm/s. The laser light under the dynamic fiber condition liquefied the adipose tissue without significant thermal injury to the peripheral tissue (around 0.5 mm). The distribution of thermal coagulation was almost consistent and circular along the diffusing applicator. A cylindrically diffusing optical applicator can be a feasible biomedical device to remove adipose tissue with 1470-nm laser light due to uniform light distribution and constant temperature elevations.

10037-38, Session PSun

Projection based multimodal imaging modality for visualization of vein and subcutaneous blood flow velocity

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Although various studies about near-infrared (NIR) imaging for vein detection and laser speckle imaging for blood flow visualization have been widely performed and shown significant advances, only few techniques have been commercialized and actually used in the fields of health care. We designed a multimodal imaging modality for both vein and blood flow visualization based on the projection method, which has a considerable advantage in terms of intuitiveness compared to the conventional displaying method using a monitor or an imaging film. In this study, we adopted projection method to visualize vein and blood flow. Although previous studies already proved the feasibility of the projection method in vein imaging, this study propose the combination of vein imaging and blood flow visualization techniques with the projection method. A near infrared (NIR) laser was used as a light source for the multimodal imaging modality. The captured image by an NIR camera were processed to visualize veins and the relative blood flow. The image pair was merged, then projected on the identical target region through a projector. The imaging modality was evaluated with the forearm veins and the optical tissue phantom. The capability to detect the position of veins and the relative blood flow, and the conformity between the vein and its projected image were evaluated. In spite of the curvature of the forearm, the projected image mostly matched to the vein position. The result presented that the imaging modality can detect the morphology of forearm veins and discriminate the blood flow variation.

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10038-1, Session 1

In vivo fluorescence imaging of an orthotopic rat bladder tumor model indicates differential uptake of intravesically instilled near-infrared labeled 2-deoxyglucose analog by neoplastic urinary bladder tissues

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Bladder cancer is one of the most expensive cancers to manage due to frequent recurrences requiring life-long surveillance and treatment. A near-infrared labeled 2-deoxy-d-glucose probe IRDye800CW-DG targeting glucose metabolism pathway has shown to enhance the sensitivity of diagnosing several types of cancers as tested on tumor models not including bladder tumor. This pilot study has explored differential uptake of intravesically administered IRDye800CW-DG in an orthotopic rat bladder tumor model. Twenty-five female Fischer rats were randomly grouped to four conditions: no-tumor-control (n=3), no-tumor-control intravesically instilled with IRDye800CW-DG (n=6), rats bearing GFP-labeled AY-27 rat bladder urothelial cell carcinoma cells and washed with saline (n=5), and rats treated with AY-27 and intravesically instilled with IRDye800CW-DG (n=11). Near-infrared fluorescence was measured from the opened bladder wall of anesthetized rat at an excitation wavelength of 750nm and an emission wavelength of 776nm, by using an in-house fluorescence imaging system. There is no statistically significant difference of the peak fluorescence intensity among the no-tumor-control bladders (n=3), the no-tumor-control bladders instilled with IRDye800CW-DG (n=6), and the GFP-labeled AY-27 treated bladders washed by saline (n=5). When compared to that of the no-tumor-control bladders instilled with IRDye800CW-DG (n=6), the fluorescence intensity of GFP-labeled AY-27 treated bladders instilled with IRDye800CW-DG and with histology confirmed neoplastic bladder tissue (n=11) was remarkably stronger (3.34 folds of the former) that was also statistically significant ($p<0.0001$). The differential uptake of IRDye800CW-DG by the neoplastic urinary bladder tissues suggests the potential for cystoscopy-adaptation to enhance diagnosis and guiding surgical management of flat urinary bladder cancer.

10038-2, Session 1

Prostate cancer diagnosis with fluorescence lifetime imaging

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More than 1 million men in the United States undergo a prostate biopsy procedure annually and approximately 200,000 men receive a diagnosis of prostate cancer. 5-10% of these men have to undergo a repeat biopsy due to insufficient tissue sampling. We are studying the utility of a multi-spectral time resolved fluorescence spectroscopy (MS-TRFS) technique for real-time prostate cancer diagnosis. The MS-TRFS imaging setup, which includes a fiberoptic set-up with a 355nm excitation light source coupled with a blue (450nm) aiming beam, was used to image ex-vivo prostatectomy specimen. The prostate tissue from 11 patients was sectioned at 2mm thickness and the fluorescence lifetime information was overlaid spatially for histology and thus, diagnostic co-registration. Initial results show that fluorescence lifetime in the 390±40nm channel, which measures collagen and elastin signatures, is longer for glandular regions than in the stromal regions. Additionally, lifetime in the 452±45nm channel, corresponding to NAD redox state, is longer in the cancerous glandular region in comparison with the normal glandular regions. Current work is focused on developing real-time quantitative algorithms to combine the fluorescence signatures from the two channels for performing prostate cancer diagnosis on biopsies.

10038-3, Session 1

Investigations on the fluorescence of urinary stones

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Urinary stones harvested from patients were under in-vitro investigation. Fluorescence measurements were performed either by taking images under blue light excitation light as well as measuring excitation-emission-matrixes. Ho:YAG-laser assisted fragmentation was performed in an aquarium set-up to derived fragmentation/dusting rates. FTIR-spectroscopy was used to identify the composition of the stone. Blue light fluorescence excitation resulted in fluorescence emission in different spectral regions spectroscopically proven by EEM-measurement. A correlation with FTIR-spectroscopy is performed. Fragmentation experiments resulted in a dependency to the applied energy/pulse and repetition rate. Using photonic techniques urinary stones could be categorized by their composition. The impact of fluorescence guidance during endoscopic laser lithotripsy will be discussed.

10038-4, Session 2

Quality control and primo-diagnosis of transurethral bladder resections with full-field OCT

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Transurethral resections are commonly used for bladder cancer diagnosis, treatment and follow-up. Cancer staging relies largely on the analysis of muscle in the resections; however, muscle presence is uncertain at the time of the resection. An extemporaneous quality control tool would be of great use to certify the presence of muscle in the resection, and potentially formulate a primo-diagnosis, in order to ensure optimum patient care. Full-field optical coherence tomography (FFOCT) offers a fast and non-destructive method of obtaining images of biological tissues at ultrahigh resolution (1μm in all 3 directions), approaching traditional histological sections. This study aimed to evaluate the potential of FFOCT for the quality control and the primo-diagnosis of transurethral bladder resections.

Over 70 transurethral bladder resections were imaged with FFOCT, shortly after excision, and before histological preparation. Image acquisition was performed in less than 5 minutes and did not affect histological preparation

quality. Side-by-side comparison with histology allowed to establish reading criteria for the presence of muscle and cancer in particular. A set of images was read blindly by urologists and pathologists who were asked to notify the presence of muscle and tumor. Results showed very good concordance between FFOCT image analysis and histology diagnosis, with over 80% accuracy.

FFOCT is a fast and non-destructive imaging technique that provides analysis results concordant with histology. Its implementation as a quality control and primo-diagnosis tool for transurethral bladder resections in the urology suite seems feasible and lets envision value for the patient.

10038-5, Session 2

Using optical coherence tomography to detect bacterial biofilm on foley catheters

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Urinary tract infections(UTI) pose a serious problem for hospital patients accounting for 33% of all hospital acquired(nosocomial) infections with indwelling foley catheters. The presence of an indwelling foley catheter provides a scaffolding for circulating planktonic bacteria to adhere to and to form microbial biofilm communities that would typically be hindered by the body's innate immune system response. It is these biofilm communities that form on the inner lumen of foley catheters that provide a reservoir of pathogenic bacteria that could dislodge or disperse from the biofilm and infect urethra or bladder mucosal tissue in the urinary tract. Current diagnostic techniques of urine microbiological cultures are lacking in differentiating asymptomatic bacteriuria and symptomatic catheter-associated urinary tract infection(CAUTI) since almost all patients with chronic indwelling catheters are almost universally bacteriuruic. There is an unmet need of a diagnostic tool to assess the difference between the pathogenesis of asymptomatic bacteriuria and CAUTI, specifically at the site of the native biofilm formation. Optical Coherence Tomography(OCT) is an emerging high resolution, minimally invasive tomographic imaging technique that has shown promise in imaging biofilm structures previously in an endoscopic setting of the airway in-vivo and in microfluidic chambers. OCT can be adapted to image various sized biological surfaces and orifices such as airway branches and blood vessels by using a variety of minature endoscopic probes. In this work OCT will be used to image biofilm structure in-vitro on the inner lumen of extravasated critical care patient's foley catheters. Scanning electron microscopy will be conducted post OCT to confirm the presence of bacterial biofilm in OCT images.

10038-6, Session 2

One to one correlation of needle based optical coherence tomography with histopathology; a qualitative and quantitative analysis in 20 prostatectomy specimens

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Prostate cancer treatment is shifting from radical to focal therapy. Instant tumor visualization on a microscopic level is crucial for clinical application of focal therapy. Optical coherence tomography (OCT) produces instant tissue visualization on a μm scale and the attenuation of OCT signal as a measure of tissue organization. The objective is to correlate qualitative and quantitative OCT analysis with histopathology.

Twenty prostates were analyzed by needle based OCT after radical prostatectomy. For precise correlation, whole mount histology slides were cut through the OCT trajectory. OCT images were classified in eight histological categories. Two reviewers independently performed assessment of the OCT images into these categories. Quantitative attenuation coefficient was used to discriminate stroma and malignant tissue. Sensitivity and specificity for detection of malignancy on OCT was calculated.

Visual analyses showed that OCT can reliably differentiate between fat, cystic and regular atrophy and benign glands. Differentiation of benign stroma and inflammation and also malignancy Gleason 3 and 4 is more difficult. Sensitivity and specificity for detection of malignancy on OCT were calculated at 77% and 75%. Quantitative analysis by means of the attenuation coefficient for differentiation between stroma and malignancy showed no significant difference (4.39 mm-1 vs. 5.31 mm-1).

Precise correlation of histology and OCT is possible and helps us to understand what we see and measure on OCT. Visual malignancy detection shows reasonable sensitivity and specificity. Our future studies focus on improving discrimination of malignancy with OCT for example by combining an extra imaging modality.

10038-7, Session 2

Prostate cancer detection with multi-angle projection imaging for combined diffuse optical tomography and transrectal ultrasound

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Existing non-invasive imaging modalities provide low sensitivity and specificity for detecting, localizing, and stratifying of prostate cancer (PC). The standard histological examination, the random biopsy using transrectal ultrasound (TRUS) imaging, is subject to sampling errors and adverse side effects. Furthermore, no reliable test for identifying high-risk patients who require immediate intervention and for addressing over-diagnosis and overtreatment exists.

As an alternative for PC diagnosis and staging, diffuse optical tomography (DOT) has been proposed. The DOT can provide information about tissue oxygenation and light scattering, which shows a strong correlation with the Gleason score. However, the deeply seated position of the prostate limits the accessibility for DOT imaging, which results in several challenging issues for DOT image reconstruction. For example, only reflectance measurement is available, and only a limited number of optical fibers can reach the target.

To overcome these issues, we designed a novel transrectal optical probe that allows for the increase of measurement points and easy co-registration between DOT and TRUS imaging. The optical probe can be combined with a commercial TRUS probe (8808e, BK ultrasound). The combined probes can rotate at a fixed position by using a reference holder. The rotation function increases the optical and TRUS data as many times as the number of angle steps. This multi-angle projection data together with a three-dimensional shape of the prostate from TRUS imaging significantly improves the quality of DOT image reconstruction. We will report on the performance of our system with phantom experiments and an ongoing clinical pilot study.

10038-8, Session 3

Marine-derived optical skin patch for wound healing and tissue regeneration

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Due to fibroproliferative responses, skin fibrosis following radiation injury, trauma, or surgery often causes a significant degree of disability and aesthetic complications in tissue. In spite of antiproliferative agents, complete prevention of the fibroproliferative scar formation is still challenging. The objective of the current study was to fabricate an optical skin patch with marine-derived biomaterials (chitosan and fucoidan) to effectively assist wound healing and to achieve inhibition of scar formation after urological surgery. In vitro cell testing was performed to identify the effect of chitosan and fucoidan on the proliferation of fibroblasts and the production of cytokines. Various concentrations of the marine materials (0.25, 0.5, 1, 2, and 4 mg/ml) were evaluated with the cultured fibroblasts. MTT was used to determine the degree of cell proliferation and added on day 1, 3, and 5. Seven male ICR mice were used to develop scar models as well as to validate the effect of the marine materials on wound healing response and fibrosis inhibition in vivo. Chitosan significantly suppressed cell proliferation with a decrease rate (2.4~13.6%) in comparison with control at 1 mg/ml. On the contrary, fucoidan hardly reduced the growth rate (5.9~17.2%). A mixture of chitosan and fucoidan effectively stimulated the wound healing process up to 64% more than the other conditions. After 7-day treatment, the scar size treated with both chitosan and fucoidan was significantly decreased (62~85%). The chitosan-fucoidan agent stimulated apoptosis of scar fibroblasts up to 50% while control (no agent) showed the least apoptosis. The proposed chitosan-fucoidan-based skin patch can be a feasible therapeutic agent for wound care management.

10038-9, Session 3

Optical clearing of vaginal tissues

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Introduction: Laser thermal remodeling of endopelvic fascia is being investigated for minimally invasive treatment of female stress urinary incontinence. Previous computer simulations of tissue thermal damage showed that a transvaginal approach is more feasible than transurethral approach. This study uses experimental studies and computer simulations to explore whether application of an optical clearing agent can further improve optical penetration depth and completely preserve vaginal wall from thermal insult during transvaginal laser subsurface remodeling of endopelvic fascia.

Methods: Several different mixtures of OCA's were tested, and 100% glycerol was found to be optimal agent for porcine vaginal tissue, ex vivo. Optical transmission studies, optical coherence tomography, reflection spectroscopy, and computer simulations (Monte Carlo light transport, heat transfer, and Arrhenius integral models of thermal damage) were performed using glycerol as an OCA.

Results: OCA produced 61% increase in transmission of 1075-nm light through vaginal wall at 37°C after 30 min. Monte Carlo simulations showed improved energy deposition in endopelvic fascia using glycerol. Without OCA, 62, 37, and 1% of energy was deposited in vaginal wall, endopelvic fascia, and urethral wall, respectively, compared with 50, 49, and 1% using OCA. Simulations incorporating OCA and contact cooling during laser irradiation resulted in 0.5 mm increase in treatment depth, allowing potential thermal tissue remodeling at 3 mm depth.

Conclusions: Use of glycerol as OCA provided improved treatment depth with potential to thermally remodel endopelvic fascia while completely preserving entire 2.7-mm-thick vaginal wall from thermal insult, during minimally invasive laser treatment of female stress urinary incontinence.

10038-10, Session 3

Temperature monitoring with FBG sensor during diffuser-assisted laser-induced interstitial thermotherapy

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Temperature variations are often monitored by using sensors operating at the site of treatment during Laser-induced Interstitial Thermotherapy (LITT). Currently, temperature measurements during LITT have been performed with thermocouples (TCs). However, TCs could directly absorb laser light and lead to self-heating (resulting in an over-estimation). Fiber Bragg grating (FBG) sensors can instead overcome this limitation of the TCs due to its insensitivity to electromagnetic interference. The aim of the current study was to quantitatively evaluate the FBG temperature sensor with a K-type thermocouple to real-time monitor temperature increase in ex vivo tissue during diffuser-assisted LITT. A 4-W 980-nm laser was employed to deliver optical energy in continuous mode through a 600-μm core-diameter diffusing applicator. A goniometric measurement validated the uniform light distribution in polar and longitudinal directions. The FBG sensor showed a linear relationship (R2 = 0.995) between wavelength shift and temperature change in air and tissue along with a sensitivity of ~ 0.0114 nm/°C. Regardless of sensor type, the measured temperature increased with irradiation time and applied power but decreased with increasing distance from the diffuser surface. The temperature elevation augmented the degree of thermal coagulation in the tissue during LITT (4.0±0.3-mm at 99°C after 120-s). The temperature elevation augmented the degree of thermal coagulation in the tissue during LITT s irradiation). The FBG-integrated diffuser was able to monitor the interstitial temperature in tubular tissue (porcine urethra) real-time during laser treatment. However, the thermal coagulation thickness of the porcine urethra was measured to be 1.5 mm that was slightly thicker (~20%) than that of the bovine liver after 4-W 980-nm laser for 48 s. The FBG temperature sensor can be a feasible tool to real-time monitor the temporal development of the temperature during the diffuser-assisted LITT to treat urethral disease.

10038-11, Session 3

Photodynamic imaging and therapy in urology: unmet needs and barriers *(Invited Paper)*

Joseph C. Liao, Timothy Chang, Stanford Univ. School of Medicine (United States)

Intraoperative integration of targeted imaging technologies is an emerging area of innovation for diagnostic and therapeutic applications in urologic surgery. From the diagnostic perspective, photodynamic diagnosis (PDD) using fluorescent cystoscopy has been shown to increase detection of bladder cancer by 20% and reduce recurrence compared to standard white light cystoscopy. An important milestone has been the inclusion of enhanced cystoscopy technologies in recent clinical guidelines. There are limitations to PDD, however, as there are increased costs (specialized endoscope, light source, camera head), technological issues with oversensitivity from false-positive fluorescence, as well as limited scope of approval for the usage of the contrast agent (hexaminolevulinate, HAL). HAL has been demonstrated in therapeutic applications as well, where photodynamic therapy (PDT) relies on the creation of reactive oxygen species from activation of a photosensitizing agent such as 5-ALA or HAL on target tissue. However, the limitations in PDT again include the non-specificity of the targeting agent as well as treatment side effects (i.e. irritative voiding symptoms, bleeding). With these developments, an area ripe for innovation are the discovery of newer agents that can bind with increased specificity to cancer tissue as well as improved photosensitizing agents, that in conjunction could provide the ability to destroy cancer specific tissue with minimal side effects to noncancerous tissue. Examples in other disciplines employ the use of targeting antibodies coupled with newer generation of photosensitizers in the near-infrared spectra may be translated for urological applications.

10038-12, Session 4

Anti-reflection coated optical fibers for use in thulium fiber laser lithotripsy

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Introduction: The Thulium fiber laser (TFL) is being studied as an alternative to Holmium:YAG laser for lithotripsy. Free-space coupling of collimated fiber laser output beam into a disposable, silica fiber for ureteroscopy is limited by back-reflected light from fiber input surface, which may result in laser shutoff or damage if left unchecked. This study examines whether anti-reflection (AR) coated fibers may reduce back-reflected light to prevent laser shutoff, increase fiber optic transmission, and potentially increase laser stone ablation rates.

Methods: Fiber optic transmission and stone ablation studies were conducted comparing uncoated and AR-coated 105- and 200-?m-core fibers. TFL wavelength of 1908 nm was coupled into silica fibers, with incrementally increasing pulse energy (5-35 mJ), fixed 500-?s pulse duration, and variable pulse rates of 50-300 Hz. For each pulse rate, 100,000 pulses were also delivered through fibers to examine for potential damage as a function of time.

Results: Back-reflection at proximal fiber surface was reduced from 3.25% with uncoated fibers to ~0.06% with AR-coated fibers. For both fiber diameters, output power was stable, and no proximal fiber damage was observed after delivery of a total of 100,000 pulses at settings up to 35 mJ, 300 Hz, and 10.5 W average power. There was no significant difference in stone ablation rates between fiber diameters (105 vs. 200-?m) or bare vs. AR-coated fibers. Adverse events (e.g. laser shutdown) due to back-reflected light were not observed using AR-coated fibers.

Conclusions: AR-coated fibers reduce back-reflection, improve energy transmission, but do not improve stone ablation rates.

10038-13, Session 4

Investigations on Ho:YAG-laser induced lithrotripsy

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Laser lithotripsy is the preferred application for the destruction of ureteral and kidney stones. Clinically Ho:YAG lasers (?=2.1µm) are used due to high absorption by water to induce thermomechanical ablation. This study focussed on the investigation of different laser parameters in relation to the stone dusting efficiency. The term dusting was defined when the ablated fragments were d<1mm in diameter while fragmentation is defined to pieces of d> 1mm. The discussion about fragment-size showed advantages like reduced surgery time.

Experiments were performed using clinical available Ho:YAG laser energy transferred via a standard fibre (Ø: 365µm) onto phantom calculi (Bego-Stones of different hardness) in a water filled vessel.

Dusting can be reached most efficient by using low energy/pulse (approx. 0.5J/pulse) and repetition rate of around 40 Hz. Higher energy/pulse showed strong repulsion and thereby increased mobility, while using lower repetition rates result in longer ablation times. With regard to the hardness of the phantoms it can be derived that on soft calculi or calculi with a very rugged surface dusting can be observed less because the stone breaks into large fragments after a short time of laser application. For hard calculi the ablation process takes a much longer time compared to soft stones.

In the following will be shown that dusting and fragmentation process depends not only on the energy/pulse and repetition rate of a Ho:YAG-laser, but also there are differences between Ho:YAG-laser systems according to the dusting efficiency.

10038-14, Session 4

The effect of pulse forming on cavitation, retropulsion and ablation efficiency during Ho:YAG laser lithotripsy

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Background and Objectives: The purpose of this study was the examination of the effects of pulse forming on cavitation generation, stone retropulsion and ablation efficiency during Ho:YAG (2,1 µm) laser lithotripsy on a calculus phantom.

Material and Methods: A clinical Ho:YAG laser system was used at various pulse energies in direct contact and short distances between the fiber and the surface of the Begostone™ calculus phantoms. The laser can run in two pulse modes: a free running laser pulse and a formed laser pulse, where the energy content in the second half of the pulse is increased when the cavitation bubble has its largest extent. The whole ablation process was recorded with a high speed camera at 100.000 frames per second. Thus the generation of the cavitation bubble could be observed with sufficient temporal resolution to determine the part of energy consumed by the cavitation process. The retropulsion of single pulses was determined by measuring the motion amplitude of the phantoms after application of a single pulse. The ablation efficiency was determined by the mass loss of the phantoms after a series of pulses applied during continuous movement of the phantoms.

Results: With the formed laser pulses less energy was consumed during cavitation formation. So the fragmentation process was more effective and the cavitation induced retropulsion was less with the formed laser pulse.

Conclusion: Lower energy consumption by cavitation and less retropulsion helps the surgeon to keep contact to the stone and have nevertheless good fragmentation efficiency.

10038-15, Session 4

Thulium fiber laser lithotripsy using a muzzle brake fiber tip

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Introduction: The single-mode Thulium fiber laser (TFL) beam allows coupling of higher power into smaller fibers than multimode Holmium laser beam profile, without proximal fiber tip degradation. A smaller fiber is desirable because it provides space in ureteroscope working channel for increased saline irrigation rates and allows maximum ureteroscope deflection. However, distal fiber tip burnback increases as fiber diameter decreases. Previous studies utilizing hollow steel sheaths around recessed distal fiber tips reduced fiber burnback, but increased stone retropulsion. In this study, a novel “fiber muzzle brake” is tested for reducing both fiber burnback and stone retropulsion by manipulating vapor bubble expansion.

Methods: Thulium fiber laser lithotripsy studies were performed at 1908 nm, 35 mJ, 500 microseconds, and 300 Hz using 100-micrrometer-core fiber. The stainless steel muzzle brake tip consisted of 1-cm-long, 560-micrometer-outer-diameter, 360-micrometer-inner-diameter tube with 275-micrometer-diameter hole located 500-micrometers from distal end.

The fiber tip was recessed a distance of 250 micrometers. Stone phantom retropulsion, fiber tip burnback, and calcium oxalate stone ablation studies were performed, ex vivo.

Results: Small stones with mass of 30.6 ± 4.4 mg and 3-mm-diameter, were ablated over 1.5-mm sieve in 21 ± 3 s (n=10), without visible distal fiber tip burn-back. Reduction in stone phantom retropulsion distance was observed when using muzzle brake tips (1.3 ± 0.5 mm) versus hollow steel tips (57.2 ± 3.1 mm), and was comparable to 100-micrometer-core bare fibers (5.8 ± 2.4 mm).

Conclusion: The muzzle brake fiber tip simultaneously provided efficient stone ablation, reduced retropulsion, and minimal fiber degradation.

10038-16, Session 4

The study of laser pulse width on efficiency of Ho:YAG laser lithotripsy

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When treating ureteral calculi, retropulsion can be reduced by using a longer pulse width without compromising fragmentation efficiency (from the studies by David S. Finley et al and Hyun Wook Kang et al.). We tested the hypothesis that at a given pulse energy level, there is a limit of the laser pulse width without compromising the Ho:YAG laser lithotripsy dusting speed. In this study, a lab build Ho:YAG laser was used as the laser pulse source, with pulse energy from 0.2J up to 3.0 J, and electrical pump pulse width from 150 us up to 1000 us. The fibers used in the investigation are SureFlexTM fibers, Model S-LLF273/365, 273/365 μm core diameter fibers. Plaster of Paris calculus phantoms were ablated at different energy levels (0.2, 0.5, 1, 2, 3J) and with different number of pulses (1, 3, 10) using different electrical pump pulse width (333, 667, 1000 μs). The dynamics of the recoil action of a calculus phantom was monitored using an accelerometer; the laser pulse width was measured by a 2 μm photo diode (Thorlabs DET10D); and the laser-induced craters were evaluated with a 3-D digital microscope (Keyence VHX-900F). Bubble formation and collapse were recorded with a high-speed camera (Photron Fastcam SA5 M2 1.3). A design of experiment (DOE) was used to minimize the number of runs for the test. We determined the optimal Ho:YAG lithotripsy laser pulse settings regarding the pulse energy and pulse width to achieve maximal dusting and minimal retropulsion. More detailed investigation on the optimal conditions for dusting of other stone samples and the fiber size effect will be conducted as a future study.

10038-17, Session 5

Optical monitoring of kidney oxygenation and hemodynamics using a miniaturized near-infrared sensor

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Background: Primary renal graft dysfunction can occur in the early postoperative period following allograft renal transplant as a result of acute tubular necrosis, acute rejection, drug toxicity, and vascular complications. Successful treatment of graft dysfunction requires early detection, accurate diagnosis and prompt medical and surgical interventions. The current noninvasive methods for diagnosis of graft dysfunction are not sensitive or specific and often result in the late diagnosis of the condition. Near-infrared spectroscopy (NIRS) is an established optical method that monitors changes in tissue hemodynamics and oxygenation in real time. We report the feasibility of direct renal NIRS monitoring to detect renal tissue ischemia and hypoxia in an animal model.

Methods: In an anesthetized pig, a customized spatially resolved (SR) NIRS sensor was directly placed and fixed over the surgically exposed kidney. Renal perfusion and oxygenation were monitored before, during and after three sets of renal artery ligation and reperfusion.

Results: Upon ligation of the renal artery and vein, total hemoglobin (THb) and oxygenated hemoglobin (O2Hb) started to decline while deoxygenated hemoglobin (HHb) rose. Immediately after releasing the artery and vein ligation O2Hb and THb rose to a higher baseline level while HHb returned to its original baseline measurement. This pattern was similar in all three trials.

Conclusions: We have shown that a miniaturized NIRS sensor can detect renal ischemia and tissue hypoxia induced by renal artery ligation in a pig model. A modification of our optical method may contribute to early detection of renal vascular complications and graft dysfunction.

10038-18, Session 5

Multimodal fiber-probe spectroscopy for the diagnostics and classification of bladder tumors

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The gold standard for the detection of bladder cancer is white light cystoscopy, followed by an invasive biopsy and pathological examination. Tissue pathology is time consuming and often prone to sampling errors. Recently, optical spectroscopy techniques have evolved as promising techniques for the detection of neoplasia. The specific goal of this study is to evaluate the application of combined auto-fluorescence (excited using 378 nm and 445 nm wavelengths) and diffuse reflectance spectroscopy to discriminate normal bladder tissue from tumor at different stages and grades. The fluorescence spectrum at both excitation wavelengths showed an increased spectral intensity in tumors with respect to normal tissues. Reflectance data indicated an increased reflectance in the wavelength range 610 nm - 700 nm for different grades of tumors, compared to normal tissues. The spectral data were further analyzed using principal component analysis for evaluating the sensitivity and specificity for diagnosing tumor. The spectral differences observed between various grades of tumors provides a strong genesis for the future evaluation on a larger patient population to achieve statistical significance. This study indicates that a combined spectroscopic strategy, incorporating fluorescence and reflectance spectroscopy, could improve the capability for diagnosing bladder tumor as well as for differentiating tumors in different stages and grades.

10038-19, Session 5

Raman spectroscopy for prostate cancer detection and characterization

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Prostate cancer is the most frequent diagnosed cancers among men. When prostate cancer occurs, the cancer does not result in only one or few localized malignant tumor, but is generally spread within the whole prostate.

In order to counteract the very high level of heterogeneities exhibited by prostate tissues, we developed a method for high-resolution co-registration of Raman spectroscopy with prostate cancer diagnosis.

Raman spectra were acquired on fresh ex vivo prostate within 2 hours after radical prostatectomy using a multi-wavelength hand-held contact probe. After the measurements, the prostate was reintegrated to the usual pathological workflow: formalin fixated and paraffin embedded (FFPE), and prepared for microscope histopathological analyses. The precise reconstruction of the prostate slice with hematoxylin & eosin (H&E) tissue allows the spatial correlation of the measured area (0.2 mm2) with the correspondent histopathological information, for point-by-point diagnosis determination. The tissue was classified into groups (normal/cancer) and subgroups according to the percentage of benign glands, stroma or cancer.

Different machine learning algorithms were tested to classify the spectra with increasing levels of categorization. Preliminary results showed that Raman spectroscopy is capable of detecting prostate cancer with an accuracy >90%. In addition, high percentages of stroma (vs. glands) have been correlated with spectral signature of collagen, which is the main constituent of extracellular matrix.

10038-20, Session 5

Orthotopic AY-27 rat bladder urothelial cell carcinoma model presented an elevated methemoglobin proportion in the increased total hemoglobin content when evaluated in vivo by single-fiber reflectance spectroscopy

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In vivo single-fiber reflectance spectroscopy (SfRS) was performed on an orthotopic AY-27 rat bladder urothelial cell carcinoma model to explore potential spectroscopic features revealing neoplastic changes. AY-27 bladder tumor cells were intravesically instilled in four rats and allowed to implant and grow for one week, with two additional rats as the control. A total of 107 SfRS measurements were taken from 27 sites on two control bladders and 80 from four AY-27 treated bladders. The spectral profiles obtained from AY-27 treated bladders revealed various levels of a methemoglobin (MetHb) characteristic spectral feature around 635nm. A multi-segment spectral analysis method estimated concentrations of five chromophore compositions including oxyhemoglobin, deoxyhemoglobin, MetHb, lipid and water. The total hemoglobin concentration ([HbT]), the MetHb proportion in the total hemoglobin and the lipid volume content showed possible correlations. The 80 measurements from the AY-27 treated bladders could separate to three sub-sets according to the MetHb proportion. Specifically, 72 were in subset 1 with low proportion (5.3%< [MetHb]<7%), 6 in subset 2 with moderate proportion (7%< [MetHb]<30%), and 2 in subset 3 with significant proportion (>30%). When grouped according to [MetHB], the [HbT] increased from 368 μMol of subset 1 to 488uMol of subset 2 to 541 μMol of subset 3, in comparison to the 285uMol of the control. The increased total hemoglobin and the elevation of MetHb proportion may signify angiogenesis and degradation in hemoglobin oxygen-transport. Additionally, the lipid volume content decreased from 2.58% in the control to <0.2% in the tumor groups, indicating disruption of sub-epithelium tissue architecture.

10038-21, Session 6

Vaginal hemodynamic changes during sexual arousal in a rat model by diffuse optical spectroscopy

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Not only men suffer from sexual dysfunction, but the number of women who have sexual dysfunction rises. Therefore, it is necessary to develop an objective diagnostic technique to examine the sexual dysfunction of female patients, who are afflicted with the disorders. For this purpose, we developed a diffuse optical spectroscopy (DOS) probe to measure the change of oxy-, deoxy-, and total hemoglobin concentration along with blood flow from vaginal wall of female rats. A cylindrical stainless steel DOS probe with a diameter of 3 mm was designed for the vaginal wall of rats which consisted of two lasers (785 and 850nm) and two spectrometers with a separation of 2 mm. A thermistor was placed on the top of the probe to measure the temperature change from vaginal wall during experiments. A modified Beer-Lambert's law is utilized to acquire the changes of oxy-, deoxy-, and total hemoglobin, and blood flow information is obtained by diffuse speckle contrast analysis technique. For the experiments, Sprague Dawley (~400 g) female rats were divided into two groups (control and vaginal dryness model). Vaginal oxygenation, blood flow and temperature were continuously monitored before and after sexual around induced by apomorphine. After the measurement, histologic examination was performed to support the results from DOS probe in the vaginal wall. The hemodynamic information acquired by the DOS probe can be utilized to establish an objective and accurate standard of the female sexual disorders.

10038-22, Session 6

Optical monitoring of testicular torsion using a miniaturized near infrared spectroscopy sensor

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Introduction: Testicular torsion is an acute scrotal condition in children and adolescents. Accurate and fast diagnosis of this condition is of great importance. Currently, Doppler ultrasound is the preferred diagnostic method. Ultrasound is not readily available in all centers and may delay surgical treatment.

In this study, a rat model was used to examine the feasibility and sensitivity of a spatially-resolved near infrared spectroscopy (SR-NIRS) system with a custom-made miniaturized optical sensor probe to detect and study changes in testicular hemodynamics and oxygenation during three degrees of induced testicular torsion and after detorsion.

Methods: Eight anesthetized rats (sixteen testes) were studied using the SR-NIRS with a miniaturized optical probe applied directly on the surgically exposed testis during 360, 720 and 1080 degrees of torsion followed by detorsion. Oxygenated, deoxygenated and total hemoglobin were studied pre and post manipulations.

Results: NIRS monitoring does reflect acute testicular ischemia and hypoxia upon inducing torsion episodes, and tissue reperfusion-reoxygenation after detorsion. In all cases, rhythmic changes in NIRS signals were observed before applying torsion episodes. The signals were disappeared after first 360 degrees of torsion and did not appear after detorsion.

Conclusions: This animal study indicates that SR-NIRS monitoring of the testes with a miniaturized sensor is a feasible and sensitive optical method to detect testicular ischemia and hypoxia immediately after torsion and testicular reperfusion upon detorsion. This study offers the potential for SR-NIRS using a miniaturized sensor to be explored as for rapid and noninvasive means of detecting testicular torsion in children.

10038-23, Session 6

Discrimination of Malignant and Benign Kidney tissue with 1064 nm dispersive Raman spectroscopy

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Kidney cancer affects 65,000 new patients every. As computerized tomography became ubiquitous, the number of small, incidentally detected renal masses increased. About 6,000 benign cases are misclassified radiographically as malignant and removed surgically. Raman spectroscopy (RS) has been widely demonstrated for disease discrimination, however intense near-infrared auto-fluorescence of certain tissues (e.g kidney) can present serious challenges to bulk tissue diagnosis. A 1064nm excitation dispersive detection RS system demonstrated the ability to collect spectra with superior quality in tissues with strong auto-fluorescence. Our objective is to develop a 1064 nm dispersive detection RS system capable of differentiating normal and malignant renal tissue. We will report on the design and development of a clinical system for use in nephron sparing surgeries. We will present pilot data that has been collected from normal and malignant ex vivo kidney specimens using a benchtop RS system. A total of 93 measurements were collected from 12 specimens (6 Renal Cell Carcinoma, 6 Normal). Spectral classification was performed using sparse multinomial logistic regression (SMLR). Correct classification by SMLR was obtained in 78% of the trials with sensitivity and specificity of 82% and 75% respectively. We will present the association of spectral features with biological indicators of healthy and diseased kidney tissue. Our findings indicate that 1064nm RS is a promising technique for differentiation of normal and malignant renal tissue. This indicates the potential for accurately separating healthy and cancerous tissues and suggests implications for utilizing RS for optical biopsy and surgical guidance in nephron sparing surgery.

10038-24, Session PSun

Novel ureteroscope illumination designs

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Introduction: Limitations of current ureteroscope illumination configurations include the presence of shadows and hot spots in images, which is further degraded by stone debris during laser lithotripsy, and may result in a decrease in stone ablation efficiency, increase in surgical operation time, and potential collateral tissue trauma as well. Previous studies have reported accidental ureteral trauma and tissue perforation from Nitinol basket wires during Holmium laser lithotripsy, due in part to poor visibility. Although saline irrigation is routinely used during ureteroscopy to flush stone dust and debris and improve visibility, sub-optimal illumination may compound these problems.

Methods: By moving away from conventional single and double point source illumination geometries and towards a ring configuration, the resulting illumination is more uniform in both axes, reducing shadows and increasing depth discrimination. Uric acid and calcium oxalate based stones were chosen for illumination and reflection spectroscopy. Porcine ureters were used as soft tissue samples for comparison.

Results: The percent difference in reflection between ureter and stones was greater than 40% for the wavelength ranges of 470-540 nm, and 600-700 nm, making these spectral regions most suitable for high contrast illumination, possibly through narrow band imaging techniques via multiple laser sources and/or optical filters.

Conclusions: These improved ureteroscope illumination designs and approaches may potentially reduce current complications due to limited visibility during laser lithotripsy, and hence increase patient safety.

10038-25, Session PSun

Miniature ureteroscope tip designs for use in thulium fiber laser lithotripsy

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Introduction: A miniature ureteroscope has the potential to eliminate the need for full anesthesia and dilation, increase patient comfort and safety during laser lithotripsy via ureteroscopy, and significantly reduce hospital costs via an office based procedure.

Methods: A prototype, 4.5 Fr (1.5-mm-OD), five channel ureteroscope tip model was developed, housing a 200-μm-ID central channel for insertion of small, 100-μm-core optical fibers and four surrounding channels, each with 510-μm-ID for instrumentation, irrigation, imaging, and illumination, respectively. Imaging was conducted using commercially available, 3k, 6k, and 10k pixel count miniature flexible borescopes with 0.4, 0.6, and 0.9 mm outer diameters, respectively.

Results: Common ureteroscopy instruments (including optical fibers, guidewires, and stone retrieval baskets) were inserted through ureteroscope tip's working channels to demonstrate feasibility. Low irrigation rates (3.9 ± 0.2 ml/min) were measured, due to the smaller working channels, revealing the need for manual pump-assisted irrigation (37.9 ± 10.5 ml/min) during potential clinical use. The 3k pixel borescope with integrated illumination was inserted through prototype ureteroscope tip unimpeded, and successfully demonstrated the ability to differentiate between hard tissues (e.g. kidney stones) and soft tissues (e.g. ureter wall), for visibility and safety. However, the 6k pixel borescope provided the most optimal solution for miniature ureteroscopy based on both image quality and instrument diameter.

Conclusions: With further development, combined use of miniature endoscopes and small optical fibers, may enable office based Thulium fiber laser lithotripsy procedures, eliminating the need for full anesthesia and dilation, increasing patient comfort and safety, and significantly reducing hospital costs.

10038-26, Session PSun

Enhancement of photoacoustic image contrast assisted with marine-oriented biocompatible astaxanthin

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A great interest has existed in biological functions of marine-oriented astaxanthin (AXT) and its potential applications such as nutraceutical, antioxidant, and anticancer agent, prevention of diabetes, cardiovascular diseases, and photothermal therapy. However, application of AXT for imaging modality has not been explored yet. The aim of the current study was to evaluate the feasibility of AXT as photoacoustic contrast agents for mapping urinary bladder tumors. AXT strongly absorbed a visible wavelength of 532 nm, and upon light absorption, the material generated strong photoacoustic signals. Both silicon tubing phantoms and ex vivo bladder tissues were tested at various concentrations (up to 5 mg/ml) of AXT to quantitatively explore variations in PA responses. The experimental results in phantom showed that PA signal amplitudes increased linearly with the AXT concentrations (threshold detection = 0.31 mg/ml). Similarly, tissue injected with AXT exhibited approximately 16-fold signal enhancement in comparison to saline at the wavelength of 532 nm. In addition, 3D image reconstruction of the artificial bladder tumors obviously visualized the margin of tumor and quantitatively measured the tumor volume. AXT can be a feasible biocompatible contrast agent for PAI to detect bladder tumors.

10038-27, Session PSun

Enhanced heating effect during astaxanthin-assisted photothermal therapy on epithelial tumors

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Astaxanthin (AXT) is a xanthophyll carotenoid, derived from natural resources that can efficiently act on molecular targets, and its potential applications in vitro and in vivo models such as an antioxidant and anticancer agent and prevention of diabetes and cardiovascular diseases. This study addressed the feasibility of AXT to enhance heating effect during photothermal treatments (PTT) on rabbit eyes tumor in vivo. A green light laser system operating at a wavelength of 532 nm was employed to induce thermal necrosis in the rabbit models. 9 rabbit models were divided into three groups: one group injected with 300 μl AXT at concentration of 300 μg/ml, one group treated with laser only, and the other one used as control. A thermal camera was used to record temperature development during laser exposure in real-time. In addition, tumor volume was also measured at each single days after treatment for all groups. The experimental results showed that AXT-assisted laser enhanced the temperature increase of 17 oC after treatment of tumor within 4 mins at the fluence of 0.1221 W/cm2, in comparison with laser irradiation only (power = 0.38 W and beam diameter = 9.95 mm). The relative variations in tumor volume confirmed that the tumors treated with AXT and laser completely disappeared after a period of 14 days, but the tumors for laser and control groups still remained. Due to selective light absorption and biocompatibility, the AXT-assisted laser treatment could serve as an effective method to treat cancer tissue.

10038-28, Session PSun

IR788-loaded sIPNs as potential tumor targeting agent for dual-functional photoacoustic imaging and photothermal therapy

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Photoacoustic imaging (PAI) represents a hybrid, non-invasive imaging techniques for tumor diagnosis due to high resolution, excellent sensitivity, and 3D image construction. However, most diseases are hardly detected by PAI due to their optical transparency at the near infrared region. It is essential to develop exogenous photoacoustic contrast agents for producing specific signals that can target the diseased tissue. The current study presents a biocompatible organic dyes IR788 loaded sIPNs as a novel photoabsorbing agent for dual-functional PA imaging and photothermal therapy (PTT) due its strong near-infrared absorption and high efficiency of photothermal conversion. A diode laser system (808 nm) was used to induce temperature increase in bladder tissue. All the samples were irradiated for 3 mins at a power density of 0.3 W/cm2. A group of silicon tubing phantoms (N=4) was injected with various concentrations (i.e. 0, 6, 8, and 10 w/w) of IR788, and tumor-bearing mice were locally injected with IR 788 at concentration of 10% w/w. Both samples were tested to quantitatively explore variations in PA responses. The PA image contrast was enhanced up to 80% and 40% in the phantom and in vivo experiments, respectively. In vitro PTT experiment indicated that the maximum increase of tissue temperature treated with IR788 was 35 °C, which was 3-fold higher than that without IR788. The results of the current study demonstrated the feasibility of the IR788 dye for cancer detection and treatment.

10038-29, Session PSun

Quantification of endogenous fluorophores and redox ratio using fluorescence spectroscopy and excitation emission matrix for prostate cancer diagnosis

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Optical biopsy is a series of rapid non-invasive or minimally invasive techniques used in biomedical and clinical field. Fluorescence spectroscopy is one of those techniques that can be used for cancer diagnosis due to its ability to reveal the changes in endogenous fluorophores by measuring the autofluorescence of tissue. Such changes may reflect morphological and metabolic level changes related to cancer development. An excitation emission matrix (EEM) is a two-dimensional fluorescence fingerprint of a sample and considered to be more robust than other types of fluorescence spectra such as emission spectra using a single wavelength excitation. In this study, autofluorescence spectra of fresh frozen normal and cancerous human prostate tissues were acquired using a Perkin-Elmer LS-55 fluorescence spectrometer. EEM were generated using the autofluorescence signal for subsequent analysis. The EEM data were analyzed using multivariate data analysis algorithms such as non-negative matrix factorization. The nonnegative spectral components were retrieved and attributed to the native fluorophores such as collagen, reduced nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide (FAD) in tissues. The retrieved loading weights of the components were used to estimate the relative concentrations of the native fluorophores and the redox ratio ([FAD]/[NADH] or [FAD]/([FAD]+[NADH])), which were then used to distinguish normal and cancerous tissues. A classification algorithm such as support vector machine (SVM) are used to separate normal and cancerous tissue samples. The efficacy of the separation is evaluated using the statistical measures including sensitivity, specificity, accuracy, and area under curve (AUC) of receiver operating characteristic (ROC).

10038-30, Session PSun

Laparoscopic prototype for optical sealing of renal blood vessels

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Introduction: Energy-based, radiofrequency and ultrasonic devices currently provide rapid sealing of blood vessels during laparoscopic procedures. We are exploring infrared lasers as an alternate energy modality for vessel sealing, capable of generating less collateral thermal damage. Previous studies demonstrated feasibility of sealing vessels in an open in vivo porcine model using 1470-nm laser. However, the initial prototype was designed for testing in open surgery and featured tissue clasping and light delivery mechanisms incompatible with laparoscopic surgery. In this study, a laparoscopic prototype similar to devices currently in surgical use was developed, and performance tests were conducted on porcine renal blood vessels, ex vivo.

Methods: The 5-mm-outer-diameter laparoscopic prototype featured a traditional Maryland jaw configuration. Laser energy was delivered through 550-micrometer-core-diameter optical fiber and side-delivery from lower jaw, with beam measuring 18-mm-length x 1.2-mm-width. The 1470-nm diode laser delivered 68 W with 3 s activation time. A total of 69 fresh porcine renal vessels with mean diameter of 3.3±1.7 mm were tested, ex vivo.

Results: Vessels smaller than 5-mm-diameter were consistently sealed (48/51) with burst pressures greater than malignant hypertension blood pressure (180 mmHg), averaging 1038±474 mmHg. Vessels larger than 5 mm were not consistently sealed (6/18), yielding burst pressures of 174±221 mmHg. Thermal damage zone from center of seal measured less than 1 mm.

Conclusions: A novel optical laparoscopic prototype with Maryland jaw configuration consistently sealed vessels less than 5-mm-diameter with minimal thermal spread. Further in vivo studies are planned to test performance across a variety of vessels and tissues.

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10039-1, Session 1

Micro-optical coherence tomography imaging of cochlear cells and nerve fibers

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Despite hearing loss's sweeping prevalence across the globe, little is known about how specific defects in the sensory organ for hearing, the cochlea, affect auditory perception in living humans. This void in our understanding of the peripheral auditory system results primarily from the fact that the cochlea's fragility, small size, complex 3D configuration, and deeply embedded position preclude visualization of its interior with standard clinical imaging technologies (e.g. CT and MRI), which are not capable of accessing the cochlea's interior or resolving details at the necessary micron-scale. We recently demonstrated the ability of micro-optical coherence tomography (μOCT), a non-invasive, low-coherence interferometric imaging technique that improves upon its predecessor (OCT) in resolving capability and depth of focus, to reveal microanatomy and physiological mechanisms involved in atherosclerosis and mucociliary clearance; here, we demonstrate this technology's ability to resolve intracochlear structures that are critical to the hearing mechanism, such as bundles of neuronal fibers, hair cells, and supporting cells in the cochlea's organ Corti. 3D volumetric reconstruction of raw image stacks enabled unique visualization of the data, such as through endoscopic perspective 'fly through' videos and virtual sectioning across any plane of interest. The present acquisition and analysis methods demonstrate significant improvements to those currently applied in clinical OCT imaging, and display intracochlear anatomy in novel and clinically informative ways. Our findings are the first to demonstrate μOCT's ability to resolve intracochlear anatomy in situ, and thus motivate further investigation into μOCT's potential utility as a tool in otologic research and clinical practice.

10039-2, Session 1

Extratympanic imaging of middle and inner ear structures of the mouse and rat model using optical coherence tomography

Se-Joon Oh, Soo-Keun Kong, Soo-Keun Wang, Pusan National Univ. Hospital (Korea, Republic of); Hong Ki Kim, Minkyu Kim, Hyunjong Kim, Jeonghun Choi, Heetae Kim, Koh Young Technology, Inc. (Korea, Republic of); Jeon Yeob Jang, Pusan National Univ. Hospital (Korea, Republic of)

Background and Objective : Optical Coherence-Tomography (OCT) has been used in other fields for obtaining high-resolution cross-sectional images of the tissue. The goal of this study was to investigate whether OCT provides information about the middle and inner ear microstructures in both rats and mice by extratympanic approach.

Materials and Methods: Six BALB/c mice and Sprague Dawley rats were enrolled to the experiment, and to acquire an image of the entire tympanic membrane, the auricle and cartilaginous external auditory canal were removed, the swept-source OCT system was tested to identify the middle and inner ear microstructures.

Results: It was possible to image through the tympanic membrane extratympanically and into the middle ear cavity involving several middle ear structures in both rats and mice. We could also obtain the inner ear images through the otic capsule and into the cochlea in the mice by extratympanic approach. However, the bulla should be removed to provide the inner ear structural images in the rats. The whole cochlea of the apical, middle and basal turn could be visualized and the bony thickness of the otic capsule covering the cochlea could also be measured simultaneously.

Conclusions: OCT is a promising technology to noninvasively assess middle ear and inner ear microanatomy in both mice and rats. These findings are meaningful because there were no previous report to describe the middle and inner ear structure of the rat looking by extratympanically.

10039-3, Session 1

The morphological analysis of optically cleared cochlea using optical coherence tomography

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In this study, we monitored the optical clearing effects by immersing ex vivo guinea pig cochlea samples in ethylenediaminetetraacetic acid (EDTA) to study the internal microstructures in the morphology of guinea pig cochlea. The imaging limitations due to the guinea pig cochlea structures were overcome by optical clearing technique. Subsequently, the study was carried out to confirm the required approximate immersing duration of cochlea in EDTA-based optical clearing to obtain the best optimal depth visibility for guinea pig cochlea samples. Thus, we implemented a decalcification-based optical clearing effect to guinea pig cochlea samples to enhance the depth visualization of internal microstructures using swept source optical coherence tomography (OCT). The obtained nondestructive two-dimensional OCT images successfully illustrated the feasibility of the proposed method by providing clearly visible microstructures in the depth direction as a result of decalcification. The most optimal clearing outcomes for the guinea pig cochlea were obtained after 14 consecutive days. The quantitative assessment results verified the increase of the intensity as well as the thickness measurements of the internal microstructures. Following this method, difficulties in imaging of internal cochlea microstructures of guinea pigs could be avoided. The obtained results verified that the depth visibility of the decalcified ex vivo guinea pig cochlea samples was enhanced. Therefore, the proposed EDTA-based optical clearing method for guinea pig can be considered as a potential application for depth-enhanced OCT visualization.

10039-4, Session 1

In vivo and in vitro analysis of pathogenic structures affixed to the tympanic membrane during chronic otitis media infection

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When performing OCT imaging of human subjects with chronic otitis media (OM), the presence of additional infection-related structures affixed to the internal surface of the tympanic membrane (TM) are regularly observed. Previous clinical studies have identified and characterized biofilms growing on the middle ear mucosa in humans with chronic OM, and theorized their role in recurrent OM infections. However, detection of these biofilms by means of invasive sampling in clinical practice is impractical. In this work, we seek to image, identify, and characterize potential middle ear biofilms affixed to the internal mucosal surface of the TM, both in vivo and in surgically recovered in vitro samples.

A total of 41 pediatric subjects were observed and imaged intraoperatively under general anesthesia while undergoing tympanostomy tube placement surgery at Carle Foundation Hospital in Urbana, IL under an IRB-approved study. Prior to and after incision into the TM (myringotomy), a handheld probe and portable OCT system quantitatively imaged and assessed the TM for the presence of middle ear biofilms, and if identified, characterized the optical properties of these structures. Biological samples were collected with surgical tooling from the imaging site (mucosal surface of TM), which was subsequently reimaged with OCT to confirm sample collection from the originally imaged site. In vitro analysis of the collected samples using fluorescence in-situ hybridization (FISH) and quantitative polymerase chain reaction (qPCR) techniques provided further microbiological characterization. Results demonstrate further evidence that chronic OM is linked with the presence of a middle ear biofilm, and that OCT is a reliable means to quickly and non-invasively identify middle ear biofilms affixed to the TM.

10039-5, Session 1

Multimodal optical imager for inner ear hearing loss diagnosis

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Sensorineural hearing loss (SNHL), which typically originates in cochlea of inner ear, is the most common otologic problem caused by aging and noise trauma. The cochlea, a delicate and complex biological mechanosensory transducer in the inner ear, has been extensively studied with the goal of improving diagnosis of SNHL. However, the difficult access to the cochlea and the absence of adequate tools limit physician's capability for performing accurate diagnosis of SNHL. To address this problem we investigated the use of an endoscopic multimodal optical probe that combines optical coherence tomography (OCT) and autofluorescence imaging (AFI), which enables access to the cochlear canal and thus the evaluation of the delicate structure and functionality of the hair cells. A laboratory OCT/AFI imager was first built to acquire cochlear high resolution OCT images and multispectral AFI images in multi-emission bands, specific to the key endogenous fluorophores. The imager was evaluated on a normal and hearing-impaired mouse model in vivo and a clear differentiation between the structural/molecular signatures of the normal and impaired cochlea was observed. Then a prototype endoscopic OCT/AFI imager was developed based on double-clad fiber approach and its feasibility for human use was tested using cadaveric temporal bones. Our measurements show that the multimodal endoscopic OCT/AFI imager can be used to differentiate between normal and hearing-impaired cochlea. Therefore, we believe that this technology may be used in the future to diagnose the SNHL, and guide otologic surgeries such as cochlear implant surgery.

10039-6, Session 2

Multi-modal anatomical optical coherence tomography and CT for in vivo dynamic upper airway imaging

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We describe a novel, multi-modal imaging protocol for validating quantitative dynamic airway imaging performed using anatomical Optical Coherence Tomography (aOCT). The aOCT system consists of a catheter-based aOCT probe that is employed via a bronchoscope, while a programmable ventilator is used to control airway pressure. This setup is employed on the bed of a Siemens Biograph CT system capable of performing respiratory-gated acquisitions. In this arrangement the position of the aOCT catheter may be visualized with CT to aid in co-registration. Utilizing this setup we investigate multiple respiratory pressure parameters with aOCT, and subsequently with respiratory-gated CT, on ex-vivo porcine trachea and on live, anesthetized pigs. This acquisition protocol has enabled real-time acquisition of changes in the airway geometry with simultaneous measurement of pressure under physiologically relevant static and dynamic conditions- inspiratory peak or peak positive airway pressures of 10-30 cm H2O and 20-30 breaths per minute for dynamic studies. We subsequently compare the airway cross sectional areas (CSA) obtained from aOCT and CT, specifically the change in CSA at different stages of the breathing cycle for dynamic studies and the CSA at different peak positive airway pressures for static studies. This approach has allowed us to improve our acquisition methodology and to validate aOCT measurements of the dynamic airway for the first time. We believe that this protocol will prove invaluable for aOCT system development and greatly facilitate translation of OCT systems for airway imaging into the clinical setting.

10039-7, Session 2

Thermographic imaging of facial and ventilatory activity during vocalization, speech and expiration

Krzysztof Izdebski, Pacific Voice and Speech Foundation (United States)

Ventilation, speech and singing must use facial musculature to complete these motor tasks and these tasks are fueled by the air we inhale. This motor process requires increase in the blood flow as the muscles contract and relax, therefore skin surface temperature changes are expected. Hence, we used thermography to image these effects. The system used was the thermography camera model FLIR X6580sc with a chilled detector (FLIR Systems Advanced Thermal Solutions, 27700 SW Parkway Ave Wilsonville, OR 97070, USA). To assure improved imaging, the room temperature was air-conditioned to +18° C. All images were recoded at the speed of 30 f/s.

Acquired data were analyzed with FLIR Research IR Max Version 4 software and software filters.

In this preliminary study a male subject was imaged from frontal and lateral views simultaneously while he performed normal resting ventilation, speech and song. The lateral image was captured in a stainless steel mirror. Results showed different levels of heat flow in the facial musculature as a function of these three tasks. Also, we were able to capture the exalted air jet directionality. The breathing jet was discharged in horizontal direction, speaking voice jet was discharged downwards while singing jet went upward. We interpreted these jet directions as representing different gas content of air expired during these different tasks, with speech having less oxygen than singing. Further studies examining gas exchange during various forms of speech and song and emotional states are warranted.

10039-8, Session 2

High speed digital phonoscopy of selected extreme vocalization

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We used HSDP (KayPENTAX Model 9710, NJ, USA) to capture the kinematics of vocal folds in the production of extreme vocalization used by heavy metal performers. The vibrations of the VF were captured at 4000 f/s using transoral rigid scope. Growl, scream and inhalatory phonations were recoded. Results showed that these extreme sounds are produced predominantly by supraglottic tissues rather than by the true vocal folds, which explains while these sounds do not injure the mucosa of the true vocal folds. In addition, the HSDI were processed using custom software (Vocalizer®) that clearly demonstrated the contribution of each vocal fold to the generation of the sound.

10039-9, Session 2

3-D renditions of the extreme vocalizations

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Growl, a style of extreme vocalization used for the production of bizarre and scary voice by heavy metal singes captured by HSDP is simply fascinating and shows that this sound is produced predominantly by the supraglottic structures. To enhance our understanding of how this process is accomplished. The obtained images were processed to be viewed in 3-D. The results are shown and discussed.

10039-10, Session 2

Analysis of laryngeal amyloidosis using high speed digital phonoscopy and acoustics

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Amyloidosis is an unknown pathogenic process in which abnormally folded proteins are deposited in the extracellular space as macroscopic aggregates. Laryngeal deposits of these proteins are extremely rare, but primarily cause dysphonia in patients. High Speed Digital Phonoscopy (HSDP) was used to capture the kinematics of vocal folds in a patient with laryngeal amyloidosis. Acoustic data was also recorded and both HSDP and acoustics were processed using custom Vocalizer® software to help elucidate the physiological impact of amyloids in the larynx, especially in regards to effects on the voice.

10039-11, Session 3

Indocyanine in localized chest wall tumors in rabbit by use of multimodal imaging modality: diffuse optical spectroscopy with Photoacousic tomography

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We present a study of the dynamics of optical contrast agents indocyanine green (ICG) in an squamous carcinoma in chest wall in rabbit using in vivo optical spectrometry and ex vivo photoacoustic tomography. Measurements are conducted with a combined frequency-domain and steady-state optical technique that facilitates rapid measurement of tissue absorption in the 650–1000-nm spectral region in vivo. In ex vivo, Chest walls(tumor group and normal group) were imaged by use of photoacoustic imaging and coregistered with the location of the optical probe. The absolute concentrations of indocyanine was measured simultaneously each second for approximately 10 min. The differing tissue uptake kinetics of ICG in these tumors arise and keep steady state for 20 min, while rapid washout of ICG was observed within 5 min in normal chest wall. NIR image(800nm) in ex vivo chest wall with tumor showed significant fluorescence, while there is no fluorescence in normal chest wall. Photoacoutic image using laser at 780nm showed significant signal enhancement in chest wall tumor 10 min after injection of ICG, while there was no signal enhancement in normal chest wall. Without injection of ICG, There was no signal enhancement in chest wall tumor nor normal chest wall. Multimodal tumor imagings of localized chest wall tumor would be challenging for future study to evaluate for functional status of tumors such as lung cancer and mesothelioma.

10039-12, Session 3

Diffuse reflectance spectroscopy from 400-1600 nm to evaluate tumor resection margins during head and neck surgery

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This ex vivo study evaluates the feasibility of diffuse reflectance spectroscopy (DRS) for discriminating tumor from healthy oral tissue, with the aim to develop a technique that can be used to determine a complete excision of tumor through intraoperative margin assessment.

DRS spectra were acquired on fresh surgical specimens from patients with an oral squamous cell carcinoma. The spectra represent a measure of diffuse light reflectance (wavelength range of 400-1600 nm), detected after illuminating tissue with a source fiber at 1.0 and 2.0 mm distances from a detection fiber. Spectra were obtained from 23 locations of tumor tissue and 16 locations of healthy muscle tissue. Biopsies were taken from all measured locations to facilitate an optimal correlation between spectra and pathological information.

The area under the spectrum was used as a parameter to classify spectra of tumor and healthy tissue. Next, a receiver operating characteristics (ROC) analysis was performed to provide the area under the receiver operating curve (AUROC) as a measure for discriminative power.

The area under the spectrum between 650 and 750 nm was used in the ROC analysis and provided AUROC values of 0.99 and 0.97, for distances of 1 mm and 2 mm between source and detector fiber, respectively.

DRS can discriminate tumor from healthy oral tissue in an ex vivo setting. More specimens are needed to further evaluate this technique with component analyses and classification methods, prior to in vivo patient measurements.

10039-13, Session 3

Autofluorescence lifetime imaging during transoral robotic surgery: a clinical validation study of tumor detection

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Autofluorescence lifetime spectroscopy is a promising non-invasive label-free tool for characterization of biological tissues and shows potential to report structural and biochemical alterations in tissue owing to pathological transformations. In particular, when combined with fiber-optic based instruments, autofluorescence lifetime measurements can enhance intraoperative diagnosis and provide guidance in surgical procedures.

We investigate the potential of a fiber-optic based multi-spectral time-resolved fluorescence spectroscopy instrument to characterize the autofluorescence fingerprint associated with histologic, morphologic and metabolic changes in tissue that can provide real-time contrast between healthy and tumor regions in vivo and guide clinicians during resection of diseased areas during transoral robotic surgery. To provide immediate feedback to the surgeons, we employ tracking of an aiming beam that co-registers our point measurements with the robot camera images and allows visualization of the surgical area augmented with autofluorescence lifetime data in the surgeon's console in real-time.

For each patient, autofluorescence lifetime measurements were acquired from normal, diseased and surgically altered tissue, both in vivo (pre- and post-resection) and ex vivo. Initial results indicate tumor and normal regions can be distinguished based on changes in lifetime parameters measured in vivo, when the tumor is located superficially. In particular, results show that autofluorescence lifetime of tumor is shorter than that of normal tissue ($p < 0.05$, $n = 3$).

If clinical diagnostic efficacy is demonstrated throughout this on-going study, we believe that this method has the potential to become a valuable tool for real-time intraoperative diagnosis and guidance during transoral robot assisted cancer removal interventions.

10039-14, Session 3

Analysis of medical imaging feasibility in presence of body fluids using Markov chains

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A relatively wide field of view and high resolution imaging is necessary for navigating the scope within the body, inspecting tissue, diagnosing disease, and guiding surgical interventions. As the large number of modes available in the multimode fibers (MMF) provides higher resolution, MMFs could replace the millimeters-thick bundles of fibers and lenses currently used in endoscopes.

The MMFs have promising potential in transmitting images through multiple parallel optical modes. With multiple modes, it is possible to use smaller size bundles of fibers, replace lenses, and perform high quality images. In recent years, there has been made tremendous progress on the performance of MMFs for high-resolution images. Also, this research draws upon mostly primary sources including MMFs for high-resolution imaging, flexible MMFs, and fiber optic fluorescence imaging research work. Most prior goals were to research on comprehension of spatial distortion and flexibility of the fibers.

However, attributes of body fluids and obscurants such as blood impose perennial limitations on resolution and reliability of optical imaging inside human body. To design and evaluate optimum imaging techniques that operate under realistic body fluids conditions, a good understanding of the channel(medium) behavior is necessary.

In most prior works, Monte-Carlo Ray Tracing (MCRT) algorithm has been used to analyze the channel behavior. This task is quite numerically intensive. The focus of this paper is on investigating the possibility of simplifying this task by a direct extraction of state transition matrices associated with standard Markov modeling from the MCRT computer simulations programs. We show that by tracing a photon's trajectory in the body fluids via a Markov chain model, the angular distribution can be calculated by simple matrix multiplications. We also demonstrate that the new approach produces results that are close to those obtained by MCRT and other known methods. Furthermore, considering the fact that angular, spatial, and temporal distributions of energy are inter-related, mixing time of Monte-Carlo Markov Chain (MCMC) for different types of liquid concentration is calculated based on Eigen-analysis of the state transition matrix and possibility of imaging in scattering media are investigated.

10039-15, Session 3

Feature extraction from in vivo wide-field OCT images for oral cancer detection

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Worldwide, there are over 450,000 new cases of oral cancer reported each year. Late-stage diagnosis remains a significant factor responsible for its high mortality rate (>50%). In-vivo non-invasive rapid imaging techniques, that can visualise clinically significant changes in the oral mucosa, may improve the management of oral cancer.

We present an analysis of features extracted from oral images obtained using our hand- held wide-field Optical Coherence Tomography (OCT) instrument. The images were analyzed for epithelial scattering, overall tissue

scattering, and 3D basement membrane topology. The associations between these three features and disease state (benign, pre-cancer, or cancer), as measured by clinical assessment or pathology, were determined. While scattering coefficient has previously been shown to be sensitive to cancer and dysplasia, likely due to changes in nuclear and cellular density, the addition of basement membrane topology may increase diagnostic ability- as it is known that the presence of bulbous rete pegs in the basement membrane are characteristic of dysplasia. The resolution and field-of-view of our oral OCT system allowed analysis of these features over large areas of up to 2.5mm x 90mm, in a timely fashion, which allow for application in clinical settings.

10039-16, Session 3

In vivo nasopharyngeal carcinoma staging using rapid fiber-optic Raman endoscopy

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We evaluate the diagnostic performance of a rapid simultaneous fingerprint and high wavenumber (HW) fiber-optic Raman endoscopy platform for in vivo real-time nasopharyngeal carcinoma (NPC) staging during clinical endoscopy. The integrated FP and HW Raman spectra were acquired from 26 cancerous sites from 10 NPC patients. Multivariate diagnostic algorithms based on principal component analysis (PCA) and linear discriminate analysis (LDA) yield an encouraging accuracy of ~84% for discrimination early and advanced NPC stage. This result reveal the great potential of the FP/HW Raman endoscopic technique developed for monitoring different NPC stages in vivo during routine endoscopic examination.

10039-17, Session 3

Longitudinal monitoring of the head and neck lymphatics in response to surgery and radiation

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Radiation therapy (RT) can promote anti-tumoral responses, but is also known to cause lymphatic endothelial cell apoptosis, loss of dermal lymphatics, and reduction in lymph transport to draining lymph node basins. When combined with lymph node dissection (LND), the radiogenic lymphatic disruption may possibly result in lymph stasis and dermal backflow. If not resolved, this disruption may lead to chronic inflammation, edema, fibrosis, adipose tissue deposition, and ultimately to functional deficits and disfigurement. Because the head and neck (HN) region contains 1/3 of the body's lymph nodes, lymphatic responses to cancer progression and therapy may be significant. Furthermore, it may not be surprising that lymphedema has been estimated to impact as many as 75% of HN cancer survivors three months or more after LND and RT.

In this study, we used near-infrared fluorescence imaging to longitudinally assess the lymphatics of 18 patients undergoing treatment for cancer of the oral cavity, oropharynx, and/or larynx following intraoral and intradermal injections of ICG. Patients were imaged before and after surgery, before and after fractionated RT for up to 100 weeks after treatments. Patients who underwent both LND and RT developed lymphatic dermal backflow on treated sides ranging from days after the start of RT to weeks after its completion, while contralateral regions that were not associated with LND but also treated with RT, experienced no such changes in functional lymphatic anatomies. The results show for the first time, the striking reorganization of the lymphatic vasculature and may enable early diagnosis of HN lymphedema.

10039-18, Session 4

Photodynamic therapy using hypericin in mycobacterium smegatis subconjunctivitis in rabbit

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Recently growing incidence of multi-drug resistant Non-Tuberculous Mycobacterial (NTM) infection has become important issue. This study investigated the antimicrobial effect of hypericin (HYP)-mediated photodynamic therapy (PDT) on Mycobacterium smegmatis(MS) conjunctivitis. HYP has powerful photo-oxidizing ability, tumor localization properties, and fluorescent imaging capabilities as well as minimal dark toxicity. The current study defined HYP-PDT in vivo, MS subconjunctivitis model in rabbit tested as a model for hypericin induced fluorescence and PDT. In vitro study, the mycobacterial suspension and colonies were treated with the following: HYP only, laser only ; MB and light (PDT). Twelve rabbits with Mycobacterium conjunctitis were randomly divided into three groups (HYP only, laser only, HYP and light (PDT)). In the in vitro tests at 100-150 J/cm2 with 10 um/ml HYP and 80mW output, There was no killing effect by Hypericin and 590 nm Laser separately, while relative survival rate reaches down to about 10% after PDT. Fluorescence telescopy showed localized fluorescence in tuberculosis lesion 5 hours after HYP injection. In the rabbit subconjunctivitis model, combined PDT resulted in significantly less bacterial burden (P=0.04) than in HYP only, laser only group. The phototoxicity was minimal. Phototoxicity was minimal and self-limited. This study demonstrated the effectiveness of HYP mediated PDT against Mycobacterium smegatis. PDT could be a potential alternative treatment for nontuberculous mycobacterial subconjunctival infections.

10039-19, Session 4

Photodynamic therapy of hypericin-treated laryngeal carcinoma in rabbit

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Conventional cancer therapy including surgery, radiation, and chemotherapy often are physically debilitating and largely ineffective in previously treated patients with recurrent head and neck squamous cell carcinoma (SCC). The natural photosensitizing agent, Hypericin, the may provide a less invasive

method for laser photodynamic therapy (PDT) of the recurrent head and neck malignancies. Hypericin has powerful photo-oxidizing ability, tumor localization properties, and fluorescent imaging capabilities as well as minimal dark toxicity. The current study defined hypericin PDT in vivo, laryngeal cancer model transplanted with VX2 SCC cells in rabbit tested as a model for hypericin induced tumor fluorescence and PDT via transoral laser fiberoptics. VX2 laryngeal cancer model was created in 12 rabbits via transoral tumor cell suspension injection approach. Experimental group (group A, n=6) was treated with 590 nm wavelength laser (500mw, 500J) using diffusing fiheroptics after injection hypericin 4 hours. In hypericin only control group (group B, n=3), hypericin was injected but lasering is not given. Lasering only control group (group C, n=3) was treated with laser without hypericin injection. All the tumors were responsive, the tumor volume was decreased and pathologic mucosa was restored to normal condition after photodynamic therapy. In control groups (only hypericin-treated and only lasering) The tumors were growing and all rabbit were passed out due to airway obstruction. The average survival period was increased more than two times after hypericin-treated PDT, and some small sized tumors were regressed completely (p=0.01). The results suggest hypericin-treated PDT may be useful treatment method for laryngeal cancer.

10039-20, Session 4

The mechanism for welding with a green laser: revisited

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Laser welding, by design, is based on tissue heating (to over 60°C), which bears the inherent risk of severe and irreversible collateral tissue damages. A promising alternative to photothermal laser welding is photochemical tissue bonding (PTB), where laser light (typically green, at ?=532 nm) is used to irradiate a photoactive dye to bond adjacent tissue surfaces. The formation of reactive singlet oxygen has been suggested, which subsequently generates a cascade of aggressive free radicals, and ultimately results in amino acid covalent cross-linking between proteins (collagen) inside the tissue. Remarkable is the fact, that during the procedure no significant increase in temperature was reported. Hence, PTB avoids localized tissue damage by heat. The PTB method is also faster than rejoining nerves or closing wounds with suture-based techniques. Considering the myriad of its benefits, it is surprising that laser tissue bonding has not been translated into the clinic yet. In order to apply and advance PTB, we have attempted to reproduce several published experiments, but were only partially successful with our efforts. More specifically, we have found indications that significant tissue heating occurs during the procedure. Further investigations based on a number of carefully designed control experiments using micro temperature probes and thermochromic ink gel confirmed the substantial temperature rise upon laser irradiation. Despite its limitations, we are convinced that this technique can be beneficial for clinical use, if the heating problem is addressed and properly dealt with.

10039-21, Session 4

The role of laser thermotherapy and local hemodynamic parameters in the differentiatad treatment of head and neck infantile hemangioma

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Objective: Improvement results of treatment for patients with infantile hemangioma (IH) of the head and neck.

Methods: The study comprised 1466 patients with IH of the head and neck treated between 2001 and 2016. Hemodynamic in IH and symmetrical, healthy area (control), were diagnosed with color Doppler flow mapping, thermography, transcutaneous oxygen tension measurement and spectrophotometry. Local hemodynamic (LH) was considered intense if the thickness of the IH had many vessels with a diameter greater than 1 mm, there is a pronounced, more than 1.5 degrees Celsius, hyperthermia and increase the average level of the relative volume of capillary blood filling more than 25%. Normal figures were similar to the control of LH. Intermediate parameters considered moderate increase.

Treatment options were IH laser thermotherapy, propranolol, or a combination thereof. IH normal LH, without the risk of ulceration was observed only and are not included in this study.

The first group of patients (n=705) was treated before 2010 with non-invasive and intralesional diode laser at 970 and 1060 nm. The second group (n=413) was managed with propranolol since 2010. In 2011 patients with intensive blood flow IH (n=348, group three) underwent propranolol treatment in combination with laser thermotherapy.

Results: Laser thermotherapy in the first group was effective in all cases but in patients with intensive LH (25.4%) treatment was done iteratively.

All patients of the second group were given propranolol 1,3-1,5 mg/kg/day for 8,8±2,6 month.

Tumor regress was noted in 46,8% of patients. In other patients of the second group with intensive LH propranolol this dosage of propranolol was ineffective.

Combination of propranolol and intralesional laser thermotherapy in patients with intensive LH IH (group three) decreased frequency of iterative laser interventions to 7,9% in compare with first group (p<0,01) and duration of propranolol treatment to 4,5 ±2,4 month (?<0,05). This method of treatment led to persistent involution of IH in 99,4% patient of the third group with good aesthetic result in 97,5%.

Conclusion: Treatment of head and neck IH must be differentiated. In patients with intensive blood flow IH combination of propranolol and intralesional laser thermotherapy is the method of choice.

10039-22, Session 4

Two-photon fluorescence microscopy to assess three-dimensional culture of human skin fibroblast growth on electrospun tissues in experimental otology

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Introduction: Titanium ossicular replacement prostheses have been uses in reconstructive middle ear surgery for a long time and can be regarded as state-of-the-art principle to replace the defective ossicular chain. Various causes, however, account for 4 to 6% of prosthesis failure by bending, displacement or extrusion. Bio-resorbable electrospun textiles have been used in other compartments of the human body to aid tissue growth in desired directions. The particular benefit for their use in middle ear surgery lies in fibroblast growth along the electrospun fibres into depth of tissue, which is difficult to assess.

Methods: Polylactide-co-Glycolide (PLGA) tissues were generated by

electrospinning and cultured with human skin fibroblasts (HDF). Cultured cells were measured by cell count, their growth and cell morphology on PLGA tissues measured by Two-Photon Fluorescence Microscopy with particular regard to their growing behaviour into the depth of electrospun tissue. Vitality of HDF and their affinity to 3D-PLGA tissues were compared to 2D-titanium platelets.

Results: Depending on the density of fibres, their thickness and processing of PLGA tissue after the process of electrospinning, HDF can penetrate in between tissue fibres and therefore grow in three-dimensional patterns as shown by Two-Photon Fluorescence Microscopy. Cell morphology orients itself in parts along the electrospun fibres, thereby suggesting a greater bonding strength to the adjacent tissue.

Conclusion: The introduction of bio-resorbable electrospun polymer fibres provides a promising modality to promote integration of ossicular chain prosthesis in surrounding middle ear tissue. Two-Photon Fluorescence Microscopy provides a valuable tool to adjust preparation of suitable tissue configurations.

10039-23, Session 4

Is optoacoustic stimulation a new way to compensate hearing impairment?

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As estimated by the WHO, 360 million persons in the world have disabling hearing loss [1]. Despite technical improvements, classical hearing aids that are based on mechanical energy still have multiple limitations. Additionally, electrical neural stimulation used to bypass the nonfunctional peripheral sensory organ, the cochlea [2], does not always sufficiently compensate the needs of hearing impaired patients. A new concept regarding the stimulation strategy of the auditory system is using light energy to activate the hearing organ. We discovered that pulsed green laser light, via the optoacoustic effect, can activate the peripheral hearing organ when applied not only at the inner ear level but also at the ear drum level and on any bony structures that could transmit vibrations to the inner ear. Using a monochrome laser system (532 nm) with a fixed short pulse length (10 ns), we were able to activate a wide range of frequencies of the auditory system in a guinea pig model. Additionally, pulse amplitude modulated laser input signals induced an activation of the central auditory system (auditory brainstem responses and inferior colliculus recordings) similar to sound induced activation. With this new stimulation concept we establish an alternative method for frequency specific activation of the complete audible spectrum using single monochrome laser pulses. The optoacoustic method may provide more effective and specific activation of the hearing system in various conditions associated with conductive, combined or sensorineural hearing loss.

10039-24, Session 4

Transplantation of rib cartilage reshaped with 1.56 ?m laser radiation in rabbits

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As cartilage is an ideal natural material for transplantation, its use in the ENT surgery is limited by a difficulty to get proper shape of cartilage implants. Aim of the work is to make ring-shaped cartilage implants and check their stability after laser reshaping of the cartilage and transplantation into rabbits In-Vivo. We experimented with costal cartilages of 1-2mm in thickness obtained from 3rd and 4rd ribs of a rabbit. 1.56 ?m laser (Arcuo Medical Inc.) was used for cartilage reshaping. The laser settings were established taking into account anisotropy of cartilage structure for different orientation of the implants. The reshaped cartilage implants were switched to rib cartilages of the other rabbits. The sites of switching were additional irradiated to facilitate regeneration processes. The rabbits were sacrificed in three months after surgery. The results shown that (1) all reshaped implants kept circular form, and (2) the implants were attached in the switched sites (3) pronounced signs of regeneration in the intermediate zones were observed. The prospects of the cartilage implants use in larynx stenosis surgery are discussed.

10040-1, Session 1

Longitudinal intravital imaging in the murine bone marrow

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Intravital two-photon microscopy (2PM) is the method of choice to study interactions, migration, or activation of cells of the immune and skeletal system in bone marrow (BM). Both cell types show high plasticity in normal tissue and especially during bone regeneration.

To date, 2PM in the BM is limited to a few hours in duration and depths < 150 μm. We have developed a reproducible microendoscopy technique based on GRIN lenses, which allows for longitudinal in vivo imaging in the marrow cavity.

The design of the implant for murine femurs facilitates the precise guidance of a 350 μm thin GRIN endoscope into the center of the marrow cavity. The implant is fixed with two bone screws to the right femur in a lateral approach. Thus, it allows repeated access to the BM without further surgical procedures over a 100 days and more.

Ex vivo μCT, immunofluorescence-based histology and histochemical methods were used to monitor tissue recovery after implantation and to proof that tissue homeostasis is reached within two to three weeks post surgery.

In a first approach, we repeatedly imaged B cells in CD19:RFP and followed plasma cell depletion after administration of Bortezomib in BLIMP1:GFP reporter mice. Vascularization was visualized by i.v. injection of Qdots.

Therefor, we demonstrated that this technique permits longitudinal intravital multiphoton microendoscopy in murine bone marrow (LIMB). We further plan to use this approach to study immune reactions in bone injury models and to elucidate the role of immune and bone cells in bone healing.

10040-2, Session 1

Nonlinear imaging and surgery using a Kagome HC-PCF

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The ideal tool for clinical laser surgery is a thin endoscope capable of both nonlinear imaging and ablation. Such a probe is only possible with in-line architecture where a single fiber is capable of delivering high pulse energies for surgery and excitation, and collecting light emitted from the sample. Traditional fibers either cannot transmit high pulse energies without nonlinear effects, or are limited in their bandwidth, and cannot collect emitted light. Kagome type hollow-core photonic crystal fibers, based on the inhibited coupling guidance mechanism, have shown record low losses of 70 dB/km at 780 nm and 500-600 nm. Furthermore, the hypocycloid shape core increases the laser induced damage threshold, allowing high energy pulses to propagate without damaging the fiber. Here, we design, fabricate, and test a Kagome HC-PCF and show its applicability towards endoscopic imaging and surgery. The fiber has a 7-cel core defect and a Kagome latticed cladding with 3 rings, resulting in 25 dB/km losses within the Ti-sapphire spectral range, and high transmission from 500-600 nm and above 1450 nm. We demonstrate the nonlinear imaging capabilities of the fiber (both excitation and collection) by two-photon imaging of fluorescent beads and third-harmonic generation imaging of tissue. We also show the surgical capabilities by ablating tissue phantoms as well as biological samples.

10040-3, Session 1

Lensless endoscopy using a fiber bundle and holographic imaging approach

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Endoscopic imaging requires the use of focusing optics and when combined with a point imaging technique such as optical coherence tomography the integration of a mechanical component is also necessary in order to perform scanning of the light beam over the tissue of interest. Consequently, these elements add size, weight and complexity to the instrument. An imaging approach that does not rely on the use of lenses or scanning of the light beam is holography, in which the interference between a reference and object wave is recorded. We have chosen this imaging approach using a numerical reconstruction of the image in combination with a fiber bundle to provide a lensless endoscope that furthermore does not require scanning of the light beam. A resolution target and biological sample were successfully imaged whereas improvements of the contrast were achieved by sweeping the laser for speckle reduction via wavelength diversity and averaging of multiple images. The same system has furthermore been used for optical coherence tomography imaging by using different signal processing steps to perform depth resolved holographic 3D-imaging. However, imaging with a fiber bundle remains challenging, due to the unwanted effects occurring when light travels through a fiber bundle, such as cross-talk and multimoding. Consequently, the performance of different fiber bundles distinguishable from each other in terms of core size, numerical aperture and flexibility has been investigated and compared.

10040-4, Session 1

Differential Structured Illumination Microendoscopy for in vivo imaging of molecular contrast agents and cervical dysplasia

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Fiber-optic microendoscopes have shown promise as an imaging tool capable of visualizing molecular contrast agents used to study disease

in vivo. The small size and flexibility of fiber-bundle probes make them ideal for in vivo use. However, image contrast can be severely limited when imaging highly scattering tissue. Optical sectioning techniques such as confocal or structured illumination can improve image contrast in microendoscopes by rejecting out-of-focus light generated in highly scattering tissue. However, optical sectioning techniques can reduce imaging speed or require complex opto-mechanical components to be installed on the distal end of the fiber-bundle. Here we present differential structured illumination microendoscopy (DSIMe) capable of performing structured illumination imaging in a fiber-optic microendoscope. Sectioning can be performed at video rates without the need for opto-mechanical components attached to the distal end of the fiber-bundle. Improved axial response of DSIMe is demonstrated using an optical phantom and we show image contrast enhancement in highly scattering mouse tissue imaged ex vivo. We also demonstrate contrast enhancement using DSIMe to image cervical tissue in vivo in patients diagnosed with cervical adenocarcinoma in situ and an improved ability to identify cellular changes associated with neoplasia.

10040-5, Session 1

Evaluation of computational endomicroscopy architectures for minimally-invasive optical biopsy

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Coherent fiber-optic bundles are often used in minimally-invasive medical imaging probes. However, these components provide only a small number of resolvable points (around 30,000 individual fibers in a 1 mm diameter bundle), and suffer from “missing data” (due to the space between individual fibers). We are addressing these limitations by applying concepts from the compressed sensing (CS) field, to perform fiber bundle based imaging with more resolvable points than fibers physically present in the bundle.

A digital micromirror device generates coded binary mask patterns which are introduced into the optical train either conjugate to the object/image planes (termed “Image Plane Coding, IPC”), or conjugate to a Fourier/aperture plane (termed “Aperture Plane Coding, APC”). The number of mask elements (micromirrors) imaged onto each fiber is termed the “undersampling factor” and determines the factor by which the pixel count in reconstructed images is increased over the native fiber bundle resolution.

We have imaged binary and grayscale optical test targets in the IPC configuration and experimentally demonstrated that CS reconstruction using Nesterov’s proximal gradient method was able to recover high resolution features, using undersampling factors up to 16x (JP Dumas et al., Optics Express 2016). We have recently assembled a platform which enables direct experimental comparison between IPC and APC configurations. This presentation will report our latest findings on image reconstruction accuracy for IPC and APC computational imaging, as well as initial demonstration of CS imaging through fiber optic bundles.

10040-6, Session 1

1.6 mm high-NA field-corrected epifluorescence endomicroscope with a chip-on-the-tip of 400x400 pixel resolution and a single-fiber illumination for brain research and later medical applications

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Minimally invasive in-vivo one-photon-fluorescence endomicroscopy has attracted a tremendous interest in recent years, especially in brain research. A miniaturized size of the probes is mandatory for a minimal destruction of tissue. One way is to use small diameter relay optics as GRIN singlets to access deep brain tissues in small animals in combination with larger miniature microscopes outside of the organ. This approach, however, is not suitable for medical applications.

Here, we demonstrate a flexible stand-alone endomicroscope with an outer diameter of 1.6 mm and a rigid length of 6.7 mm that enables surgeons or biologists to image hardly accessible tissue regions in-vivo in epi-fluorescence mode. The device overcomes spatial limitations of state-of-the-art objectives by combining gradient index lenses and tiny spherical lenses with an extremely miniaturized chip-on-the-tip camera of 400x400 pixel resolution. A large field of view of 220 μm in diameter and a numerical aperture of 0.7 allow the observation of subcellular features and assure a bright and high-contrast image. The endomicroscope excites epi-fluorescence in the tissue at a wavelength below 490 nm utilizing a single multi-mode fiber and captures the polychromatic image at 30 Hz video-rate above 510 nm.

We present the capabilities of the probes by in-vivo imaging of genetically modified mouse brains and fluorescent beads. In the future, the development can support surgeons at endoscopic interventions or early cancer detection at hardly accessible areas or help biologists performing intravital microscopic research as long-time observations of brain regions in freely moving animals.

10040-7, Session 2

Optical characterization and polarization calibration for rigid endoscopes

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Polarization measurements give orthogonal information to spectral images making them a great tool in the characterization of environmental parameters in nature. Thus, polarization imagery has proven to be remarkably useful in a vast range of biomedical applications. One such application is the early diagnosis of flat cancerous lesions in murine colorectal tumor models, where polarization data complements NIR fluorescence analysis. Advances in nanotechnology have led to compact and precise bio-inspired imaging sensors capable of accurately co-registering multidimensional spectral and polarization information. As more applications emerge for these imagers, the optics used get more complex and can potentially compromise the original polarization state of the incident light. Here we present a complete optical and polarization characterization of three rigid endoscopes of size 1.9mm x 10cm (Karl Storz, Germany), 5mm x 30cm and 10mm x 33cm (Olympus, Germany), used in colonoscopy for the prevention of colitis-associated cancer. Characterization results show that the telescope optics act as retarders and effectively depolarize the linear component. These incorrect readings can cause false-positives leading to an improper diagnosis. In this paper, we offer a polarization calibration scheme for these endoscopes based on Mueller calculus. By modeling from training data these individual optics as real-valued Mueller matrices we are able to successfully reconstruct the initial polarization state acquired by the imaging system. As a result of our analysis we find linear polarimeter sensors subpar for the use of optics with retarder elements and a full polarimeter sensor is proposed.

10040-8, Session 2

Minimally invasive optical biopsy for oximetry

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An adequate supply of oxygen is of critical importance to the healthy function of cells and tissues. Many inflammatory diseases such as rheumatoid arthritis result in affected tissue becoming hypoxic. Currently, devices such as Clark electrodes and oxygen quenching probes allow for bulk measurement of oxygen saturation (SO2) in vivo. However, localised quantification of SO2 remains a challenge for vasculature which is not optically accessible. To date, we have developed a novel multispectral imaging system and oximetry technique, employing blue wavelengths which provide sufficient contrast for accurate oximetry in the small blood vessels of the microvasculature. We report results of previous proof-of-concept experiments in surgically exposed tendons of mice in vivo, which validate the sensitivity of the illumination scheme and analysis technique to changes in SO2 with inflammation. Average SO2 for controls was 94.80 ± 6.98 % (N = 6), and 83.98 ± 13.46 % for mice with inflamed tendon tissue (N = 6). We also report recent progress which extends this technique to minimally invasive oximetry, using a microendoscopic triplet lens (? = 500 ?m) and multispectral fibre illumination scheme. The microendoscope and multiple illumination fibres are secured within a modified hypodermic needle using UV-cured epoxy for penetration through tissue. Employing a microendoscope in this way circumvents the need for surgical exposure of tendon microvasculature. This will allow for longitudinal studies of SO2 over the course of disease progression, providing a greater understanding of the link between hypoxia and inflammatory disease.

10040-9, Session 2

Spectral and lifetime endoscopic measurements using one and two-photon excitation

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Current surgical biopsy takes several days to be analyzed by anatomopathologists, who provide analysis report to the surgeon a few days after the surgical intervention. Moreover, tissue biopsies are often associated with patient discomfort as they are highly invasive. In addition, the lack of precise guidance often leads to inaccuracies in the selection of tissue regions for biopsy. Eliminating this time consuming process of conventional biopsy is a clinical need, as well as increasing the accuracy of tissue diagnostics and patient comfort. We propose to address these needs by developing a multimodal nonlinear endomicroscope that allow real-time optical biopsies during surgery. It will provide immediate diagnosis without removal of tissue and assist in the choice of optimal therapy means. This instrument will combine several means of contrast: non-linear fluorescence, second harmonic generation, reflectance, fluorescence lifetime and spectral analysis. Multimodality is crucial for reliable and comprehensive analysis of tissue. In this work we present a first study of spectral and fluorescence lifetime measurements taken by this endomicroscopic system based on a new homemade optical fiber. This new homemade double-clad micro structured optical fiber insures visible and near infrared excitation. Spectral and lifetime measurements were accomplished using one and two photons excitation on freshly and fixed extracted human brain tissue. Results of these endomicroscopic measurements were compared to a bench top microscope measurments and to the literature.

10040-10, Session 2

In vivo multiplexed molecular endoscopy of esophageal cancer in an orthotopic rat model with topically applied SERS nanoparticles

Yu Wang, Soyoung Kang, Jonathan T. C. Liu, Univ. of Washington (United States)

Esophageal cancer patients often present with advanced metastatic disease at the time of diagnosis, resulting in poor survival and cure rates. The biological investigation and detection of esophageal cancers could be facilitated with a multiplexed molecular endoscopic technology to screen for the molecular changes that precede and accompany the onset of cancer. Surface-enhanced Raman scattering (SERS) nanoparticles (NPs) have the potential to improve cancer detection and investigation through the sensitive and multiplexed detection of cell-surface biomarkers. Here, we demonstrate that the topical application and endoscopic imaging of biomarker-targeted SERS NPs enables the rapid detection of tumors in an orthotopic rat model of esophageal cancer, in which various “flavors” of SERS NPs may be identified by their unique spectral signatures. A multiplexed cocktail of antibody-conjugated SERS NPs was topically applied on the lumenal surface of the rat esophagus to target multiple biomarkers, and a miniature spectral endoscope featuring rotational scanning and axial pull-back was employed to comprehensively image the NPs bound on the lumen of the esophagus. Ratiometric analyses of specific binding vs. nonspecific accumulation of SERS NPs enabled the visualization of tumors and the quantification of biomarker expression levels in agreement with immunohistochemistry and flow cytometry validation data.

10040-11, Session 3

Feasibility study of Tethered Capsule Endomicroscopy (TCE) deployment in the small intestine

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Environmental enteric dysfunction (EED) is a poorly understood disease of the small intestine that causes nutrient malabsorption in children, predominantly from low and middle income countries. The clinical importance of EED is neurological and growth stunting that remains as the child grows into adulthood. Tethered capsule endomicroscopy (TCE) has the potential to improve the understanding of EED and could be used to determine the effectiveness of EED interventions. TCE in the adult esophagus and the duodenum has been demonstrated for Barrett`s esophagus and celiac disease diagnosis, respectively. While adult subjects can independently swallow these capsules, it is likely that infants will not, and, as a result, new strategies for introducing these devices in young children aged 0.5-2 years need to be investigated. Our first approach will be to introduce the TCE devices in infants under the aid of endoscopic guidance. To determine the most effective method, we have tested endoscopic approaches for introducing TCE devices into the small intestine of living swine. These methods will be compared and contrasted to discuss the most effective means for endoscopic tethered capsule introduction into the small intestine.

10040-12, Session 3

Optimizing the villi visualization by tethered capsule OCT endomicroscopy for comprehensive imaging of human duodenum

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Celiac disease (CD) affects around 1% of the global population and can cause serious long-term symptoms including malnutrition, fatigue, and diarrhea, amongst others. Despite this, it is often left undiagnosed. Currently, a tissue diagnosis of CD is made by random endoscopic biopsy of the duodenum to confirm the existence of microscopic morphologic alterations in the intestinal mucosa. However, duodenal endoscopic biopsy is problematic because the morphological changes can be focal and endoscopic biopsy is plagued by sampling error. Additionally, tissue artifacts can also an issue because cuts in the transverse plane can make duodenal villi appear artifactually shortened and can bias the assessment of intraepithelial inflammation. Moreover, endoscopic biopsy is costly and poorly tolerated as the patient needs to be sedated to perform the procedure.

Our lab has previously developed technology termed tethered capsule OCT endomicroscopy (TCE) to overcome these diagnostic limitations of endoscopy. TCE involves swallowing an optomechanically-engineered pill that generates 3D images of the GI tract as it traverses the lumen of the organ via peristalsis, assisted by gravity. In several patients we have demonstrated TCE imaging of duodenal villi, however the current TCE device design is not optimal for CD diagnosis as the villi compress when in contact with the smooth capsule's wall. In this work, we present methods for structuring the outer surface of the capsule to improve the visualization of the villi height and crypt depth. Preliminary results in humans suggest that new TCE capsule enables better visualization of villous architecture, making it possibly to comprehensively scan the entire duodenum to obtain a more accurate tissue diagnosis of CD.

10040-13, Session 3

Design of tethered capsule endomicroscopy systems for Barrett's Esophagus screening

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Optical coherence tomography (OCT) is an imaging technology that provides depth-resolved images by using interferometry to measure the optical delay of backscattered light from a sample. This technology has been shown in prior studies to be capable of accurately diagnosing Barrett's Esophagus (BE), a metaplastic change that conveys an increased risk of developing esophageal adenocarcinoma (EAC). Tethered capsule endomicroscopy (TCE) using OCT is a technology developed in our lab where a tethered, opto-mechanical pill is swallowed and obtains 10 μm resolution cross-sectional OCT images of the entire esophageal wall as it traverses the esophagus via peristalsis.

Our first generation TCE device used a rotary junction (RJ) and a driveshaft to convey torque that rotated optics within the capsule to scan the beam along the esophageal wall. The cost of this driveshaft-based device was high, potentially hindering widespread adoption of this technology for screening. In this work, we have developed a next-generation TCE device that is significantly less expensive in both fixed and disposable costs while producing better and more quantitative image data. In the new design, we replaced the expensive RJ and driveshaft by an inexpensive cell-phone micro-motor costing less than $10. Implementing this second-generation device was challenging because inexpensive cell-phone motors are not designed to reliably attain constant velocity rotation. This issue was mitigated through a combination of pulse-width drive modulation, appropriate loading of the motor, and by using image-based control-loop feedback. In this presentation, we will describe these technical solutions in detail and will present human data from our inexpensive cell-phone motor-based TCE device. Based on our results with this new technology, we believe that the cost of TCE devices can be lowered to the level that would be required to implement widespread OCT screening for BE.

10040-14, Session 3

In vivo imaging of human esophagus with SECM half-inch tethered endoscopic capsule

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Spectrally encoded confocal microscopy (SECM) is a high-speed reflectance microscopy technology that has the potential to image large areas of the esophagus at cellular resolution in vivo. Previously, we demonstrated in vivo imaging of the human esophagus with SECM endoscopic devices and showed that SECM can visualize key cellular features associated with healthy and diseased esophagus. Recently, we have developed a half-inch-diameter SECM tethered endoscopic capsule (HITEC) tailored to image unsedated subjects with suspected and diagnosed Barrett's esophagus. In this paper, we present results from a pilot clinical study imaging the human esophagus with the SECM HITEC devices. The HITEC device had an outer diameter of 12.7 mm and achieved lateral resolution of 1.2 μm and axial resolution of 18 μm. In this pilot study, we have enrolled three human subjects to date. Each subject was first asked to swallow the SECM HITEC device. Once the HITEC device was positioned inside the esophagus, the integrated SECM optics rotated at 3 rotations/sec while continuously acquiring confocal images. The HITEC device traversed between the gastroesophageal junction and the proximal esophagus four times to acquire multiple large-area confocal images. In the middle of imaging, subjects swallowed a mixture of vinegar and maple syrup that was used to enhance the nuclear contrast. All three subjects successfully swallowed the HITEC device and there were no complications. SECM images clearly showed squamous epithelial cell nuclear detail. In conclusion, the preliminary results from this study show that the SECM HITEC device can be safely used in human subjects for visualizing nuclei of esophageal tissue.

10040-15, Session 3

Tethered SECM endoscopic capsule for the diagnosis of eosinophilic esophagitis

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Eosinophilic Esophagitis (EoE) is an inflammatory disease caused by inhaled or ingested food allergies, and characterized by the infiltration of eosinophils in the esophagus. The gold standard for diagnosing EoE is to conduct endoscopy and obtain multiple biopsy specimens from different portions of the esophagus; an exam is considered positive if more than 15 eosinophils per high power field (HPF) in any of the biopsies. This method of diagnosis is problematic because endoscopic biopsy is expensive and poorly tolerated and the esophageal eosinophil burden needs to be monitored frequently during the course of the disease. Spectrally encoded confocal microscopy (SECM) is a high-speed confocal microscopy technology that can visualize individual eosinophils in large microscopic images of the human esophagus, equivalent to more than 30,000 HPF. Previously, we have demonstrated that tethered capsule SECM can be conducted in unsedated subjects with diagnosed EoE. However, speckle noise and the relatively low resolution in images obtained with the first capsule prototypes made it challenging to distinguish eosinophils from other cells. In this work, we present a next-generation tethered SECM capsule, which has been modified to significantly improve image quality. First, we substituted the single mode fiber with a dual-clad fiber to reduce speckle noise. A gradient-index multimode fiber was fusion spliced at the tip of the dual-clad fiber to increase the effective numerical aperture of the fiber from 0.09 to 0.15, expanding the beam more rapidly to increase the illumination aperture at the objective. These modifications enabled the new SECM capsule to achieve a lateral resolution of 1.8 μm and an axial resolution of 16.1 μm, which substantially improves the capacity of this probe to visualize cellular features in human tissue. The total size of the SECM capsule remained 6.75 mm in diameter and 31 mm in length. We are now in the process of testing this new SECM capsule in humans. Early results using this new SECM capsule suggest that this technology has the potential to be an effective tool for the diagnosis of EoE.

10040-16, Session 4

In vivo microscopy of white blood cells using spectrally encoded flow cytometry

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White blood cells (WBC) analysis is an important part of the complete blood count, providing good indication of the patient's immune system status. The most common types of WBCs are the neutrophils and lymphocytes that comprise approximately 60% and 30% of the total WBC count, respectively; differentiating between these cells at the point of care would assist in accurate diagnosis of the possible source of infection (viral or bacterial) and in effective prescription of antibiotics. In this work, we demonstrate the potential of spectrally encoded flow cytometry (SEFC) to non-invasively image WBC in human patients, allowing morphology characterization of the main types of WBCs. The optical setup includes a broadband light that was diffracted and focused onto a single transverse line within the cross section of a small blood vessel at the inner patient lip. Light backscattered from the tissue was measured by a high-speed spectrometer, forming a two-dimensional reflectance confocal image of the flowing cells. By imaging at different depths into vessels of different diameters, we determine optimal imaging conditions (i.e. imaging geometry, speed and depth) for counting the total amount of WBCs and for differentiating between their main types. The presented technology could serve for analyzing the immune system status at the point of care, and for studying the morphological and dynamical characteristics of these cells in vivo.

10040-17, Session 4

Measuring sickle cell morphology in flow using spectrally encoded flow cytometry

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During a sickle cell crisis in sickle cell anemia patients, deoxygenated red blood cells may change their mechanical properties and block small blood vessels, causing pain, local tissue damage and even organ failure. Measuring these cellular structural and morphological changes is important for understanding the factors contributing to vessel blockage and developing an effective treatment. In this work, we use spectrally encoded flow cytometry for confocal, high-resolution imaging of flowing blood cells from sickle cell anemia patients. A wide variety of cell morphologies were observed by analyzing the interference patterns resulting from reflections from the front and back faces of the cells' membrane. Using numerical simulation for calculating the two-dimensional reflection pattern from the cells, we propose an analytical expression for the three-dimensional shape of a characteristic sickle cell and compare it to a previous from the literature. In vitro spectrally encoded flow cytometry offers new means for analyzing the morphology of sickle cells in stress-free environment, and could provide an effective tool for studying the unique physiological properties of these cells.

10040-18, Session 4

Forward-viewing spectrally encoded endoscopy

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Miniaturized endoscopes with diameters less than 1 mm, have the potential to greatly reduce trauma and complications during internal imaging and treatment procedures. Spectrally encoded endoscopy (SEE) is one promising miniature endoscopy technique that can capture high quality video streams through a very small diameter, flexible device. Previously, SEE devices have been primarily side-viewing because forward-viewing required more complex optics. In this paper, we present a forward-viewing SEE system and probe. Broadband light was delivered to illumination optics through a single mode fiber. Inside the illumination optics, light was focused by a miniature GRIN lens (diameter = 350 μm), reflected by a mirror surface (angle-polished surface of a 500-μm-diameter glass rod), and incident on a miniature grating The incident angle on the grating was carefully chosen so that the shortest wavelength of the spectrum propagated along the optical axis of the illumination optics. Two-dimensional illumination was accomplished by rotating the illumination optics at a rotation speed of 15 rps using a miniature torque coil. Reflected light from the sample was collected by a circular array of 16 multimode fibers. On the distal side, the circular fiber array was rearranged to illuminate a linear fiber array and light from the linear array was detected by a custom spectrometer with a tall-pixel camera (1024 pixels). The size of the final device inclusive of the detection fiber array was 1.3 mm in diameter. The rigid length was 6.2 mm. The SEE probe achieved a field angle of 52° and the total number of effective pixels was 71,000. SEE videos showed that this technology enables endoscopic-like visualization of biological and non-biological samples. Results from this preliminary development demonstrate that high-definition endoscopic imaging is feasible though a miniature forward-viewing SEE device.

10040-19, Session 4

Single-fiber approach for color spectrally encoded endoscopy

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Spectrally encoded endoscopy (SEE) is a miniature endoscopic technology that can potentially acquire high-definition images of internal organs through a hair-thin probe. Since SEE expends wavelength information for encoding spatial information, images obtained with SEE lack color information. A previously demonstrated color SEE approach used three light beams incident on a single grating at different angles. However, translation of this approach into a miniature SEE probe has been challenging due to the need for three fibers. In this paper, we present a simpler optics design that uses a single illumination fiber for conducting color SEE. The new approach uses high-order grating diffraction, where 5th order diffraction of blue spectrum, 4th order of green, and 3rd order of red overlap with each other on the tissue. Therefore each point on the tissue is illuminated by three distinctive wavelengths. The grating (800 lpmm) was carefully designed to i) have high diffraction efficiencies of the diffraction orders used for SEE imaging and ii) minimize diffraction efficiencies of neighboring diffraction orders, which might generate flare or ghosts. Optimal design results showed that the grating can achieve an average diffraction efficiency of 50% and diffraction efficiency of neighboring orders can be reduced to 1/15th of that of the imaging orders. Theoretical estimation showed that the color SEE probe (diameter = 500 μm) will achieve a field angle of 50º and provide 358k, 229k, and 129k pixels/frame for blue, green, and red spectra, respectively.

10040-20, Session 4

Ultraminiature video-rate forward-view spectrally encoded endoscopy

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As one of the smallest endoscopes that have been demonstrated, the spectrally encoded endoscope (SEE) shows potential for the use in minimally invasive surgeries. While the original SEE is designed for side-view applications, the forward-view (FV) scope is more desired by physicians for many clinical applications because it provides a more natural navigation.

Several FV SEEs have been designed in the past, which involve either multiple optical elements or one optical element with multiple optically active surfaces. Here we report a complete FV SEE which comprises a rotating illumination probe within a drive cable, a sheath and a window to cover the optics, a customized spectrometer, hardware controllers for both motor control and synchronization, and a software suite to capture, process and store images and videos.

In this solution, the dispersion element, i.e. the grating, is designed such that the slightly focused light after the focusing element will be dispersed by the grating, covering forward view angles with high diffraction efficiencies. As such, the illumination probe is fabricated with a diameter of only 275 ?m. The two-dimensional video-rate image acquisition is realized by rotating the illumination optics at 30 Hz. In one finished design, the whole scope diameter including the window assembly is 1.2 mm.

10040-21, Session 5

Extended depth of focus tethered capsule OCT endomicroscopy for upper gastrointestinal tract imaging

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Endoscopy, the current standard of care for the diagnosis of upper gastrointestinal (GI) diseases, is not ideal as a screening tool because it is costly, necessitates a team of medically trained personnel, and typically requires that the patient be sedated. Endoscopy is also a superficial macroscopic imaging modality and therefore is unable to provide detailed information on subsurface microscopic structure that is required to render a precise tissue diagnosis. We have overcome these limitations through the development of an optical coherence tomography tethered capsule endomicroscopy (OCT-TCE) imaging device. The OCT-TCE device has a pill-like form factor with an optically clear wall to allow the contained opto-mechanical components to scan the OCT beam along the circumference of the esophagus. Once swallowed, the OCT-TCE device traverses the esophagus naturally via peristalsis and multiple cross-sectional OCT images are obtained at 30-40 ?m lateral resolution by 7 ?m axial resolution. While this spatial resolution enables differentiation of squamous vs columnar mucosa, crucial microstructural features such as goblet cells (~10 ?m), which signify intestinal metaplasia in BE, and enlarged nuclei that are indicative of dysplasia cannot be resolved with the current OCT-TCE technology.

In this work we demonstrate a novel design of a high lateral resolution OCT-TCE device with an extended depth of focus (EDOF). The EDOF is created by use of self-imaging wavefront division multiplexing that produces multiple focused modes at different depths into the sample. The overall size of the EDOF TCE is similar to that of the previous OCT-TCE device (~ 11 mm by 26 mm) but with a lateral resolution of ~ 8 ?m over a depth range of ~ 2 mm. Preliminary esophageal and intestinal imaging using these EDOF optics demonstrates an improvement in the ability to resolve tissue morphology including individual glands and cells. These results suggest that the use of EDOF optics may be a promising avenue for increasing the accuracy of OCT-TCE for the diagnosis of upper GI diseases.

10040-22, Session 5

Endoscopic micro-OCT for cellular imaging

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Optical coherence tomography (OCT) is an advanced imaging technology for acquiring three-dimensional microstructure of biological samples. In general, it has a limitation to visualize cellular or sub-cellular structures due to its insufficient spatial resolution about 10 ?m. Micro-optical coherence tomography (μ-OCT), which has a spatial resolution up to about 1 μm using a broadband light source and a high power objective lens, enables clear visualization of cellular and sub-cellular features of biological samples. Here, we present a miniaturized endoscopic μ-OCT system, which provides three-dimensional image of luminal tissue with cellular-level resolution, using 1-mm diameter optics and optical rotary joint. This fully fiber-based endoscopic μ-OCT system provides enhanced optical and mechanical stability. The performance of the system was evaluated by imaging various phantoms, including micro-beads. Finally, three-dimensional images of blood vessel were acquired ex vivo using the endoscopic μ-OCT. These images demonstrated clear visualization of the cellular features of normal and plaque lesion of the blood vessel. We anticipate that the endoscopic μ-OCT system will promote clinical and pre-clinical studies of various diseases, such as cardiovascular disease and gastrointestinal disease.

10040-24, Session 5

Miniature multimodal endoscopic probe based on double-clad fiber

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Optical coherence tomography (OCT) can obtain light scattering properties with a high resolution, while photoacoustic imaging (PAI) is ideal for mapping optical absorbers in biological tissues, and ultrasound (US) could penetrate deeply into tissues and provide elastically structural information. It is attractive and challenging to integrate these three imaging modalities into a miniature probe, through which, both optical absorption and scattering information of tissues as well as deep-tissue structure can be obtained. Here, we present a novel side-view probe integrating PAI, OCT and US imaging based on double-clad fiber which is used as a common optical path for PAI (light delivery) and OCT (light delivery/detection), and a 40 MHz unfocused ultrasound transducer for PAI (photoacoustic detection) and US (ultrasound transmission/receiving) with an overall diameter of 1.0 mm. Experiments were conducted to demonstrate the capabilities of the integrated multimodal imaging probe, which is suitable for endoscopic imaging and intravascular imaging.

10040-25, Session 5

Clinical utility of ultrahigh speed endoscopic optical coherence tomography and angiography in gastroenterology

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We have recently developed an ultrahigh speed endoscopic swept source OCT system with 600kHz axial scan rate that achieves >10x imaging speed compared to commercial endoscopic OCT systems. In combination with distal scanning micromotor devices, this technology enables acquisition of OCT volumes with high voxel density and minimal motion artifacts. Using a 3.2mm diameter imaging probe, this system allows depth resolved en face visualization of tissue microstructure, i.e. pit patterns, as well as the ability to perform OCT angiography (OCTA) with ~20um transverse and ~8um axial resolutions. We have collected co-registered volumetric OCT and OCTA datasets from patients with a variety of upper and lower gastrointestinal (GI) tract diseases; including Barrett's esophagus and associated dysplasia, esophageal adenocarcinoma, gastric antral vascular ectasia (GAVE), chronic radiation proctitis, colonic hyperplasia and adenoma, and inflammatory bowel disease (IBD). We present representative cross-sectional and en face OCT and OCTA images that shows characteristic hallmarks of these pathologies. Furthermore, we report our diagnostic studies on Barrett's dysplasia. OCT features indicative of neoplasia as well as results of blinded reading of the datasets by multiple readers will be presented. Preliminary results indicate that endoscopic OCT and OCTA can be a viable adjunct to other advanced endoscopic imaging techniques such as narrow band imaging, chromoendoscopy and confocal laser endomicroscopy.

10040-26, Session 6

High resolution microphotonic needle for endoscopic imaging

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GRIN (Graded index) lens have revolutionized micro endoscopy enabling deep tissue imaging with high resolution. The challenges of traditional GRIN lenses are their large size (when compared with the field of view) and their limited resolution. This is because of the relatively weak NA in standard graded index lenses.

Here we introduce a novel micro-needle platform for endoscopy with much higher resolution than traditional GRIN lenses and a FOV that corresponds to the whole cross section of the needle. The platform is based on polymeric (SU-8) waveguide integrated with a microlens micro fabricated on a silicon substrate using a unique molding process. Due to the high index of refraction of the material the NA of the needle is much higher than traditional GRIN lenses. We tested the probe in a fluorescent dye solution (19.6 μM Alexa Flour 647 solution) and measured a numerical aperture of 0.25, focal length of about 175 μm and minimal spot size of about 1.6 μm. We show that the platform can image a sample with the field of view corresponding to the cross sectional area of the waveguide (80x100 μm2). The waveguide size can in principle be modified to vary size of the imaging field of view. This demonstration, combined with our previous work demonstrating our ability to implant the high NA needle in a live animal, shows that the proposed system can be used for deep tissue imaging with very high resolution and high field of view.

10040-27, Session 6

Focus scanning with feedback-control for fiber-optic nonlinear endomicroscopy

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Fiber-optic nonlinear endomicroscopy represents a strong promise to enable translation of nonlinear microscopy technologies to in vivo applications, particularly imaging of internal organs. Two-dimensional imaging beam scanning has been accomplished by using fiber-optic scanners or MEMS scanners. Yet nonlinear endomicroscopy still cannot perform rapid and reliable depth or focus scanning while maintaining a small form factor. Shape memory alloy (SMA) wire had shown promise in extending 2D endoscopic imaging to the third dimension. By Joule heating, the SMA wire would contract and move the endomicroscope optics to change beam focus. However, this method suffered from hysteresis, and

was susceptible to change in ambient temperature, making it difficult to achieve accurate and reliable depth scanning. Here we present a feedback-controlled SMA actuator which addressed these challenges. The core of the feedback loop was a Hall effect sensor. By measuring the magnetic flux density from a tiny magnet attached to the SMA wire, contraction distance of the SMA wire could be tracked in real time. The distance was then fed to the PID algorithm running in a microprocessor, which computed the error between the command position and the current position of the actuator. The current running through the SMA wire was adjusted accordingly. Our feedback-controlled SMA actuator had a tube-like shape with outer diameter of 5.5 mm and length of 25 mm, and was designed to house the endomicroscope inside. Initial test showed that it allowed more than 300 microns of travel distance, with an average positioning error of less than 2 microns. 3D imaging experiments with the endomicroscope is underway, and its imaging performance will be assessed and discussed.

10040-28, Session 6

High-resolution image reconstruction for GRIN lens probe

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Graded-index (GRIN) lenses have been widely used for developing compact imaging devices due to the small dimensions and simple optics designs. GRIN lenses, however, have intrinsic aberration which causes a distortion of the image and thus are subject to limited resolution and blurred imaging quality. Here, we employ the high-precision wavefront measurement technique for compensation of the distortion of a GRIN lens to obtain a high-resolution and high-contrast image. In doing so, we demonstrate a high-resolution and ultra-thin endo-microscope using a GRIN. A reflection-type interferometric microscope through a GRIN lens was constructed using multiple lasers (473 nm, 532 nm, and 633 nm) as light sources. The characteristics of the aberration of the GRIN lens were measured using the digital holographic method. The distortion of the GRIN lens was removed by numerical image processing with the prior information from the pre-calibration. We apply this technique to a reflection image of biological tissues acquired by our custom-built GRIN lens probe. Consequently, a diffraction limited lateral resolution as well as improved axial resolution can be achieved. Our approach will facilitate the use of GRIN lenses for compact imaging devices without compromising optical resolution and image quality.

10040-29, Session 6

Inline fiber partial reflector common path optical coherence tomography probe

Kanwarpal Singh, Joseph A. Gardecki, Guillermo J. Tearney, Massachusetts General Hospital (United States)

Background: Dispersion imbalance and polarization mismatch between the reference and sample arm signals can lead to image quality degradation in optical coherence tomography (OCT). One approach to reduce these image artifacts is to employ a common-path geometry in fiber-based probes. In this work, we report an 800 um diameter all-fiber common-path monolithic probe for coronary artery imaging where the reference signal is generated using an inline fiber partial reflector.

Methods: Our common-path probe was designed for swept-source based Fourier domain OCT at 1310 nm wavelength. A face of a coreless fiber was coated with gold and spliced to a standard SMF-28 single mode fiber creating an inline partial reflector, which acted as a reference surface. The other face of the coreless fiber was shaped into a ball lens for focusing. The optical elements were assembled within a 560 μm diameter drive shaft, which was attached to a rotary junction. The drive shaft was placed inside a transparent sheath having an outer diameter of 800 μm.

Results: With a source input power of 30mW, the inline common-path probe achieved a sensitivity of 104 dB. Images of human finger skin showed the characteristic layers of skin as well as features such as sweat ducts. Images of coronary arteries ex vivo obtained with this probe enabled visualization of the characteristic architectural morphology of the normal artery wall and known features of atherosclerotic plaque.

Conclusion: In this work, we have demonstrated a common path OCT probe for cardiovascular imaging. The probe is easy to fabricate, will reduce system complexity and overall cost. We believe that this design will be helpful in endoscopic applications that require high resolution and a compact form factor.

10040-30, Session 6

Astigmatism corrected common path probe for optical coherence tomography

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Background

Dispersion imbalances and sample arm beam astigmatism are commonly encountered artifacts in optical coherence tomography (OCT) catheters for intraluminal imaging. In this work we developed a probe that minimizes such artifacts.

Methods

Our probe was fabricated using a single mode fiber at the tip of which a glass spacer and graded index objective lens were spliced to achieve the desired focal distance. The signal was reflected using a curved reflector to correct for astigmatism from the thin, protective, transparent sheath that surrounds the optics. The probe design was optimized using Zemax, a commercially available optical design software. Common path operation was achieved using Fresnel reflection from the tip of the focusing graded index objective lens. The performance of the probe was tested using a custom designed spectrometer-based OCT system.

Results

The probe achieved an axial resolution of 15.6 um in air and a sensitivity of 103 dB. A scattering tissue phantom was imaged to test the performance of the probe for astigmatism correction. Images of the phantom confirmed that this common-path, astigmatism-corrected OCT imaging probe had minimal artifacts in the axial and lateral dimensions.

Conclusions

In this work, we developed an astigmatism-corrected, common path probe that minimizes artifacts associated with standard OCT probes. This design may be useful for OCT applications that require high axial and lateral resolutions.

10040-23, Session PSun

Micro-OCT endoscopic probe for gastrointestinal tracts imaging

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Detection of intraepithelial neoplasia of gastrointestinal (GI) tracts requires a noninvasive in vivo imaging tool that is capable of visualizing cellular and subcellular details of epithelium such as the cell size, nuclear size / shape, and mitotic activity. In this study, we designed and fabricated a flexible and compact micro-optical coherence tomography (?OCT) probe providing a spatial resolution of 2-8 ?m in tissue. The outer diameter of the optics

is 2 mm so that it may be fit in the instrument channel of the existing GI endoscope. We adopted a common-path design in which an apodizing prism separates the input beam to a central circular beam (reference) and an annual sample beam. The probe is rotated by a rotary joint so that the side-viewing imaging beam scans circumferentially. We characterized the imaging performances of the probe by imaging phantoms and the results demonstrated that the spatial resolution was 2.3 ?m in the axial direction, 3.3-8.0 ?m in the transverse direction, and the sensitivity was 92.2 dB with the sample power of 2.58 mW. To test the capability of this endoscopic probe, we imaged the rat GI tracts ex vivo. We were able to clearly visualize the layered structures of the GI mucosa. In addition, we were able to visualize the goblet cells in the rat colon ex vivo. Further development of ?OCT endoscopic catheter may provide a new avenue for early diagnoses of the GI cancers.

10040-35, Session PSun

Dual-axis confocal microendoscopy: in vivo near-infrared imaging and identification of rat colon epithelial gaps

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Confocal microendoscopy is an essential tool for observing epithelial structure of the gastrointestinal tract (GI). Emerging studies show that increased number of epithelial gaps are observed in inflammatory bowel disease patients due to the cell shedding and impaired epithelial barrier function in GI tract. Currently, great advancements have been made with confocal microendoscopy to identify epithelial gaps. However, they are limited to providing horizontal images only or having limited imaging depth. Dual axes confocal microendoscopy may overcome this limitation and provide greater tissue penetration and improved resolution with fluorescence images. We demonstrate a near-infrared fluorescence dual-axes confocal endomicroscope with a 5.5mm outer diameter which can generate high-resolution horizontal and vertical cross-sectional images. A 3D monolithic (MEMS) mirror was used for high-speed beam scanning. The endomicroscope has a lateral and vertical field-of-view of 1000 ? 1000?m2 and 1000 x 430?m2, respectively, and can achieve resolution of 2.49?m (lateral) and 4.98?m (axial). In this work, we present in vivo images of a normal rat colon. A nonspecific contrasting agent, Indocyanine Green (ICG) was sprayed onto the colon and the endomicroscope was then used to detect near-infrared fluorescence from ICG. From the images, we observed epithelial gaps with average size of less than 6 ?m, suggesting there are missing cells in the gaps. The results convince that the dual-axes confocal endomicroscope can potentially be utilized for the early prediction of human inflammatory bowel diseases.

10040-31, Session 7

A handheld MEMS-based line-scanned dual-axis confocal microscope for early cancer detection and surgical guidance

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Considerable efforts have been recently undertaken to develop miniature optical-sectioning microscopes for in vivo microendoscopy and point-of-care pathology. These devices enable in vivo interrogation of disease as a real-time and noninvasive alternative to gold-standard histopathology, and therefore could have a transformative impact for the early detection of cancer as well as for guiding tumor-resection procedures. Regardless of the specific modality, various trade-offs in size, speed, field of view, resolution, contrast, and sensitivity are necessary to optimize a device for a particular application. Here, a miniature MEMS-based line-scanned dual-axis confocal (LS-DAC) microscope, with a 12-mm diameter distal tip, has been developed for point-of-care pathology. The dual-axis architecture has demonstrated superior rejection of out-of-focus and multiply scattered photons compared to a conventional single-axis confocal configuration. The use of line scanning enables fast frame rates (≥15 frames/sec), which mitigates motion artifacts of a handheld device during clinical use. We have developed a method to actively align the illumination and collection beams in this miniature LS-DAC microscope through the use of a pair of rotatable alignment mirrors. Incorporation of a custom objective lens, with a small form factor for in vivo application, enables the device to achieve an axial and lateral resolution of 2.0 and 1.1 microns, respectively. Validation measurements with reflective targets, as well as in vivo and ex vivo images of tissues, demonstrate that this high-speed LS-DAC microscope can achieve high-contrast imaging of fluorescently labeled tissues with sufficient sensitivity for applications such as oral cancer detection and guiding brain-tumor resections.

10040-32, Session 7

targeted sections in either xy or xz plane with dual axes confocal endomicroscope

Gaoming Li, Univ. of Michigan Health System (United States); Haijun Li, Univ. of Michigan (United States); Xiyu Duan, Univ. of Michigan Health System (United States); Quan Zhou, Juan Zhou, Kenn R. Oldham, Thomas D. Wang M.D., Univ. of Michigan (United States)

We demonstrate a dual axes confocal architecture, which can be used to collect horizontal(XY-plane) or vertical cross-sectional(XZ-plane) images for tissue. This scanner head is 5.5mm in outer diameter(OD), and integrates a 3D MEMS scanner with a compact chip size of 3.2?2.9mm2. To realize the miniaturization, there are some obstacles of the small size of 3D MEMS scanner, MEMS wire bundle, the air pressure effect for MEMS motion, the processing of parabolic mirror, and optical alignment to come over. In our probe, separation mechanical structure for optical alignment was adopted and a step shape MEMS holder was designed to deal with the difficult of MEMS wire bundle. Peptides have been demonstrated tremendous potential for in vivo use to detect colonic dysplasia. This class of in vivo molecular probe can be labeled with near-infrared (NIR) dyes for visualizing the full depth of the epithelium in small animals. To confirm our probe performance, we take use of USAF 1951 resolution target to test its lateral and axial resolution. It has lateral and axial resolution of 2.49um and 4.98um, respectively. When we collect the fluorescence imaging of colon, it shows that the field of view are 1000um?1000um (horizontal) and 1000um?430um (vertical). The horizontal and vertical cross-sectional images of fresh mouse colonic mucosa demonstrate imaging performance with this miniature instrument.

10040-33, Session 7

MEMS based side-view confocal endomicroscope for switchable horizontal and oblique plane imaging

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Tremendous advances have been made in technological development of whole body molecular imaging, including PET, SPECT, MRI, bioluminescence, and ultrasound. However, a great unmet need still exists for high resolution imaging of biological processes that occur in the epithelium, the thin layer of tissue where many important cancers originate. Confocal endomicroscopes designed with a fiber bundle are used in the clinic, but they can only create images in the horizontal plane. Imaging in the plane perpendicular to the tissue surface is also important because epithelial cells differentiate in the vertical direction. Subtle changes in normal tissue differentiation patterns can reveal the early expression of cancer biomarkers.

In this work, we present a side-viewing confocal endomicroscope that can collect images in either horizontal or oblique plane using an integrated monolithic electrostatic 3D MEMS scanner. The endomicroscope can perform sub-cellular resolution imaging in both the horizontal plane and the oblique plane with FOVs of 500 x 700 μm2 and 500 x 200 μm2. A side-viewing probe will allow optimal contact between the imaging window and the luminal wall, which makes it easy to navigate in the hollow organ. The endomicroscope is packaged into a stainless steel tube with outer diameter of 4.2 mm, which can be used for both small animal and human GI tract imaging. We demonstrate in vivo imaging of colonic dysplasia in mice, showing the endomicroscope can potentially be used for early detection and staging of colon cancer.

10040-34, Session 7

A MEMS scanner with lateral and axial scanning capability for dual axes confocal endomicroscopic in-vivo imaging

Haijun Li, Gaoming Li, Xiyu Duan, Thomas D. Wang, Univ. of Michigan (United States)

Aimed to build a dual-axes confocal endomicroscope with an outer diameter of 5.5mm for in-vivo imaging applications, an electrostatic MEMS scanner has been developed to enable two dimensional (2D) light scanning in either horizontal plane or vertical cross-sectional plane. The device has a compact structure design to match the dual axes confocal architecture in the probe without blocking the collimated light beams of excitation and collection, and a cutting-free silicon-on-insulator(SOI) micromachining process is used for the fabrication. A novel lever-based gimbal-like mechanism is employed to enable three degrees of freedom motions for lateral and axial light scanning, and its geometry is optimized for achieving large deflection with high scanning speed. Based on parametric excitation, the device can work in resonant modes. Testing result shows that, up to ±27º optical deflection angle for inner axis torsion motion with a frequency of ~4.9kHz, up to ±28.5º optical deflection angle for outer axis torsion motion with a frequency of~0.65kHz and ~360μm stroke for out-of-plane translation motion with a frequency of ~0.53kHz are achieved with <60V driving voltage. Based on these results, 2D imaging with frame rate of 5~10Hz and large field of view (1000?mx1000?m in horizontal plane and 1000?mx400?m in vertical plane) can be enabled by this scanner.

10041-1, Session 1

Monitoring real-time changes in mucus concentration during saline treatments of hBE and Calu-3 cell cultures using diffusion-sensitive OCT

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Mucus concentration (wt%) is critical to respiratory health. In cystic fibrosis, hydration of mucus by the epithelium is limited because of genetic mutations, leading to dehydrated, high wt% mucus. High wt% mucus collapses the cilia, hindering mucus transport out of the lungs, resulting in air flow obstruction and infection. We have developed a novel technique to obtain space-time resolution (0.5s?4.65μm) real-time measurements of mucus wt% in-situ using Diffusion-Sensitive OCT (DS-OCT). DS-OCT applies dynamic light scattering theory to M-mode OCT images to determine the diffusion rate (Dt) of PEG-coated gold nanorods (GNRs) in mucus on live epithelia. Pore sizes within the mucus gel are comparable to GNRs dimensions (~100nm pores vs. 84?24nm GNRs), and mucus pore sizes decrease with increasing wt%, thus Dt decreases in dehydrated mucus with higher wt%. We measure Dt of GNRs in mucus up to 3.5wt%. The plot of Dt vs. wt% is fit to a 4th order polynomial (R^2=0.998) and used to spatially map the dynamic changes in mucus wt% during hypertonic-saline (HS) and isotonic-saline (IS) treatments. DS-OCT reveals that in hBE cells, HS stimulates fluid secretion within the first minute of treatment, swelling/hydrating the mucus layer, whereas IS only moderately hydrates mucus. In Calu-3 cells, HS also stimulates mucus swelling/hydration, but more slowly than observed in hBE cells. IS responses in Calu-3 cells are similar to those observed in hBE cells. DS-OCT provides, for the first time, real-time mucus hydration and concentration measurements in-situ for saline-treatment responses that could be translated into clinical studies.

10041-2, Session 1

Characterization of neutrophil migration using high resolution micro-optical coherence tomography

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Chronic dysregulated influx of neutrophil into the airway increases neutrophil burden and augments the inflammatory processes often observed in diseases such as cystic fibrosis. The quantification of neutrophil influx is often accomplished with the use of destructive tests such as imaging cytometry and myeloperoxidase assay. However, those methods are unable to capture information about the cascade of events that precede trans-epithelium migration. In this work, we employed a high resolution micro-optical coherence tomography (μOCT) technology to perform real time imaging of neutrophil activity across airway epithelial cells grown on the underside of Transwell permeable supports. This inverted configuration allows the creation of an air-liquid interface at the apical side of the cells. The μOCT imaging technology, based on the principles of spectral-domain OCT, has a lateral and axial resolution of 2 and 1.3μm, respectively. In addition, it has an axial range of approximately 300μm and is capable of recording cross-sectional images at 40 fps. By raster scanning the illumination beam, the behavior of the neutrophils across a 3D volume can be recorded over time. Thus, this imaging modality is capable of resolving individual neutrophils and, potentially, capturing the cascading events involving neutrophil tethering, subsequent adhesion to activated epithelial cells and the ultimate passage through the epithelial cells to the air space on the apical side. As a result, not only can the amount of neutrophil migration be quantified, how neutrophils behave, organize and interact with the epithelial cells and each other can also be more closely analyzed by μOCT imaging.

10041-3, Session 1

Quantification and visualization of injury and regeneration to the ciliated epithelium using quantitative flow imaging and speckle variance optical coherence tomography

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Mucociliary flow is an important defense mechanism in the lung to remove inhaled pathogens and pollutants. A disruption of ciliary flow can lead to respiratory infections. Even though patients in the intensive care unit (ICU) either have or are very susceptible to respiratory infections, mucociliary flow is not well understood in the ICU setting. We recently demonstrated that hyperoxia, a consequence of administering supplemental oxygen to a patient in respiratory failure, can lead to a significant reduction of cilia-driven fluid flow in mouse trachea. There are other factors that are relevant to ICU medicine that can damage the ciliated tracheal epithelium, including inhalation injury and endotracheal tube placement. In this study we use two animal models, Xenopus embryo and ex vivo mouse trachea, to analyze flow defects in the injured ciliated epithelium. Injury is generated either mechanically with a scalpel or chemically by calcium chloride (CaCl2) shock, which efficiently but reversibly deciliates the embryo skin. In this study we used optical coherence tomography (OCT) and particle tracking velocimetry (PTV) to quantify cilia driven fluid flow over the surface of the Xenopus embryo. We additionally visualized damage to the ciliated epithelium by capturing 3D speckle variance images that highlight beating cilia. Mechanical injury disrupted cilia-driven fluid flow over the injured site, which led to a reduction in cilia-driven fluid flow over the whole surface of the embryo (n=7). The calcium chloride shock protocol proved to be highly effective in deciliating embryos (n=6). 3D speckle variance images visualized a loss of cilia and cilia-driven flow was halted immediately after application. We also applied CaCl2-shock to cultured ex vivo mouse trachea (n=8) and found, similarly to effects in Xenopus embryo, an extensive loss of cilia with resulting cessation of flow. We investigated the regeneration of the

ciliated epithelium after an 8 day incubation period, and found that cilia had regrown and flow was completely restored. In conclusion, OCT is a valuable tool to visualize injury of the ciliated epithelium and to quantify reduction of generated flow. This method allows for systematic investigation of focal and diffuse injury of the ciliated epithelium and the assessment of mechanisms to compensate for loss of flow.

10041-4, Session 1

Improving imaging of the air-liquid interface in living mice by aberration-corrected optical coherence tomography (mOCT)

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Failure in mucociliary clearance is responsible for severe diseases like cystic fibroses, primary ciliary dyskinesia or asthma. Visualizing the mucous transport in-vivo will help to understanding transport mechanisms as well as developing and validating new therapeutic intervention. However, in-vivo imaging is complicated by the need of high spatial and temporal resolution. Recently, we developed microscopy optical coherence tomography (mOCT) for non-invasive imaging of the liquid-air interface in intact murine trachea from its outside.

Whereas axial resolution of 1.5 μm is achieved by the spectral width of supercontinuum light source, lateral resolution is limited by aberrations caused by the cylindric shape of the trachea and optical inhomogenities of the tissue. Therefore, we extended our mOCT by a deformable mirror for compensation of the probe induced aberrations. Instead of using a wavefront sensor for measuring aberrations, we harnessed optimization of the image quality to determine the correction parameter.

With the aberration corrected mOCT ciliary function and mucus transport was measured in wild type and ?ENaC overexpressing mice, which served as a model for cystic fibrosis.

10041-5, Session 1

Visualization of ex vivo human ciliated epithelium and induced flow using optical coherence tomography

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The ciliated epithelium is important to the human respiratory system because it clears mucus that contains harmful microorganisms and particulate matter. We report the ex vivo visualization of human trachea/bronchi ciliated epithelium and induced flow characterized by using spectral-domain optical coherence tomography (SD-OCT). A total number of 17 samples from 7 patients were imaged. Samples were obtained from Columbia University Department of Anesthesiology's tissue bank. After excision, the samples were placed in Gibco Medium 199 solution with oxygen at 4°C until imaging. The samples were maintained at 36.7°C throughout the experiment. The imaging protocol included obtaining 3D volumes and 200 consecutive B-scans parallel to the head-to-feet direction (superior-inferior axis) of the airway, using Thorlabs Telesto system at 1300 nm at 28 kHz A-line rate and a custom built high resolution SDOCT system at 800nm at 32 kHz A-line rate. After imaging, samples were processed with H&E histology. Speckle variance of the time resolved datasets demonstrate significant contrast at the ciliated epithelium sites. Flow images were also obtained after injecting 10?m polyester beads into the solution, which shows beads traveling trajectories near the ciliated epithelium areas. In contrary, flow images taken in the orthogonal plane show no beads traveling trajectories. This observation is in line with our expectation that cilia drive flow predominantly along the superior-inferior axis. We also observed the protective function of the mucus, shielding the epithelium from the invasion of foreign objects such as microspheres. Further studies will be focused on the cilia's physiological response to environmental changes such as drug administration and physical injury.

10041-6, Session 1

Optical coherence tomography (OCT) imaging of airway behavior before and after bronchoconstriction

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Asthma is a chronic inflammatory disease characterized by hyperreactive airways. The symptoms of asthma are intermittent dyspnea, cough, and wheezing due to environmental triggers. To better understand this chronic condition, it is essential to visualize the healthy and impaired in vivo airway behavior. However, there is no imaging modality with sufficient spatial and temporal resolution to dynamically assess airway behavior. OCT, a non-ionizing, light-based imaging modality enables real-time visualization of the airways during dynamic breathing maneuvers. The aim of this study was to investigate the structure and function of healthy and constricted airways in dependent and non-dependent regions of the lung.

Sheep (n=3) were anesthetized and mechanically ventilated. Six dependent and 6 non-dependent airways were imaged with OCT in control and constricted (Methacholine administration) condition. OCT imaging with a 2.4 Fr (0.8 mm diameter) catheter was used to acquire circumferential cross-sectional images during dynamic maneuvers: regular tidal breathing, and in a response to a single deep inhalation and to one minute of double tidal volume ventilation.

Significantly different airway behavior was observed in control and constricted conditions in dependent versus non-dependent airway segments during the maneuvers. Dependent airways are more distensible than non-dependent airways during regular tidal breathing under normal condition, while non-dependent airways are more distensible than the dependent airway segments after a single deep inhalation maneuver in response to Methacholine. Thus OCT was able to provide valuable insight into the complex behavior of airway structure and function in a sheep model of asthma.

10041-7, Session 2

Flexible OCT needle probe for image-guided endoscopic tissue aspiration

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Lung cancer commonly metastasizes through the lymphatic system. Accurate assessment of metastasis often requires transbronchial needle aspiration (TBNA). In this procedure, a flexible needle is endoscopically inserted into the patient's airway, coupled to a small ultrasound probe. Ultrasound-guidance is used to position the needle to aspirate lymphoid tissue. However, accurate positioning of the needle is difficult, particularly for small lymph nodes or lesions. Slight misplacement of the needle tip will position it in lung parenchyma, resulting in a non-diagnostic sample. This can significantly extend the time and cost of the procedure and, in extreme cases, result in failure to identify cancer metastasis. To address this issue, we integrated an all-fiber OCT probe within a standard clinical flexible needle (19-guage, 1.1mm OD) to provide intraoperative guidance during needle aspiration. The OCT needle probe consists of a sequence of no-core fiber, GRIN fiber and angle-polished no-core fiber spliced to a length of SMF and interfaced to a 1300nm spectral-domain OCT scanner. In contrast to other designs, we include a second, parallel channel through which tissue is aspirated simultaneously with imaging. The OCT allows visualization of the tissue in which the needle is positioned during aspiration. We performed in situ imaging in a sheep airway. The probe was able to differentiate three tissue types: solid tissues, such as lymphoid tissue or muscle; lung parenchyma characterized by air-filled alveoli; and adipose. This design will improve guidance during TBNA, allowing the clinician to identify whether the needle is accurately positioned within the correct tissue type.

10041-8, Session 2

Imaging demonstration of a flexible micro-OCT endobronchial probe

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The human respiratory system is protected by a defense mechanism termed mucociliary clearance (MCC). Deficiency in MCC leads to respiratory obstruction and pulmonary infection, which often are the main causes of morbidity and mortality in diseases such as cystic fibrosis and chronic obstructive pulmonary disease (COPD). Studying key parameters that govern MCC, including ciliary beat frequency, velocity and volume of airway mucus transport, as well as periciliary liquid layer thickness are therefore of great importance in understanding human respiratory health. However, direct, in vivo visualization of ciliary function and MCC has been challenging, hindering the diagnosis of disease pathogenesis and mechanistic evaluation of novel therapeutics.

Our laboratory has previously developed a 1-μm resolution optical coherence tomography method, termed Micro-OCT, which is a unique tool for visualizing the spatiotemporal features of ciliary function and MCC. We have previously described the design of a flexible 2.5 mm Micro-OCT probe that is compatible with standard flexible bronchoscopes. This device utilizes a common-path interferometer and annular sample arm apodization to attain a sharply focused spot over an extended depth of focus.

Here, we present the most recent iteration of this probe and demonstrate its imaging performance in a mouse trachea tissue culture model. In addition, we have developed an ergonomic assembly for attaching the probe to a standard bronchoscope. The ergonomic assembly fixes the Micro-OCT probe's within the bronchoscope and contains a means transducing linear motion through the sheath so that the Micro-OCT beam can be scanned along the trachea. We have tested the performance of these devices for Micro-OCT imaging in an anatomically correct model of the human airway. Future studies are planned to use this technology to conduct Micro-OCT in human trachea and bronchi in vivo.

10041-9, Session 2

Flexible optical coherence tomography catheter for transbronchial imaging

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Lung cancer is the leading cause of cancer-related death in the United States. Early diagnosis is very critical to patient survival. Most lung cancers are accidently found in X-ray or Computed Tomography (CT). However, the majority of CT detected nodules are benign, with a false positive rate of approximately 94%. No current imaging technique has sufficient resolution or tissue contrast necessary for diagnosis. The only way to determine if the nodule is malignant or not is through biopsy. Minimal invasive techniques, such as bronchial biopsy suffer from large sampling errors, and transthoracic or surgical techniques have a higher risk of complications. Our goal is to increase the diagnostic yield of low-risk transbronchial biopsy using optical coherence tomography (OCT). Previously, we have demonstrated that OCT can reliably differentiate nodules from surrounding parenchyma with >93% accuracy. We will present our latest design of a small diameter flexible OCT catheter that fits through a standard 18 gauge transbronchial needle for imaging. OCT biopsy guidance will be accomplished by confirming the needle placement inside the nodule prior to tissue collection. We anticipate that this will result in an increased diagnostic yield of transbronchial biopsy. We have tested the safety and feasibility of our transbronchial OCT catheter in a preclinical model using artificial nodules. We will present the preliminary results of our flexible OCT catheter for increasing the diagnostic yield of transbronchial biopsy.

10041-10, Session 2

Endoscopic sensing of pH in the distal lung

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(United Kingdom) and Queen's Medical Research Institute, The Univ. of Edinburgh (United Kingdom); Sohan Seth, School of Informatics, The Univ. of Edinburgh (United Kingdom); Thomas Craven, Queen's Medical Research Institute, The Univ. of Edinburgh (United Kingdom); James M. Stone, Univ. of Bath (United Kingdom); Loulia K. Mati, The Univ. of Edinburgh (United Kingdom); Colin J. Campbell, School of Chemistry, The Univ. of Edinburgh (United Kingdom); Mark Bradley, Queen's Medical Research Institute, The Univ. of Edinburgh (United Kingdom) and The Univ. of Edinburgh (United Kingdom); Christopher K. I. Williams, The Univ. of Edinburgh (United Kingdom); Kevin Dhaliwal M.D., Queen's Medical Research Institute, The Univ. of Edinburgh (United Kingdom); Timothy A. Birks, Univ. of Bath (United Kingdom); Robert R. Thomson, Heriot-Watt Univ. (United Kingdom) and Queen's Medical Research Institute, The Univ. of Edinburgh (United Kingdom)

In healthy humans, the physiological state in the distal lung alveolar acinar units is tightly regulated by normal homeostatic mechanisms. Pulmonary abnormalities such as chronic obstructive pulmonary disease, that are characterized by recurrent cycles of inflammation and infection involving dense infiltration by myeloid derived peripheral blood cells, may result in significant perturbation of the homeostatic baselines of physiology in addition to host tissue damage. Therefore, the ability to quantify and monitor physiology (e.g. pH, glucose level, oxygen tension) within the alveolar acinar units would provide a key biomarker of distal lung innate defence. Although in vitro modeling of fundamental biological processes show remarkable sensitivity to physiological aberrations, little is known about the physiological state of the distal lung due to the inability to concurrently access the alveolar sacs and perform real-time sensing. Here we report on previously unobtainable measurements of alveolar pH using a fiber-optic optrode and surface enhanced Raman spectroscopy (SERS) and show that alveolar pH changes in response to ventilation. The endoscope-deployable optrode consisted of para-mercaptobenzoic acid functionalized 150 nm gold nanoshells located at the distal end, and an asymmetric dual-core optical fiber designed for spatially separated optical pump delivery and SERS signal collection in order to circumvent the unwanted Raman signal originating from the fiber itself. We demonstrate a ~ 100-fold increase in SERS signal-to-fiber background ratio and pH sensing at multiple sites in the respiratory acinar units of a whole ex vivo ovine lung model with a measurement accuracy of ± 0.07 pH units.

10041-11, Session 3

Mucosal and vascular changes in lung rejection *(Invited Paper)*

Atul Mehta M.D., Cleveland Clinic Lerner Research Institute (United States); Jarmanjeet Singh M.D., The Cleveland Clinic (United States)

Rejection is the 'Achilles heel' in success of any type of solid organ transplantation. Lung transplant has the least 5-year survival compared to other solid organ transplantation. It is mostly attributed to bronchiolitis obliterans syndrome (BOS) considered to be part of chronic lung allograft dysfunction (CLAD). Different types of rejection which affect lung transplantation include hyper-acute (first 24 hours), acute (humoral and T-cell mediated), and chronic or CLAD. Mucosal and vascular changes in these rejections are of a great importance in developing new diagnostic and treatment modalities. Hereby we summarize these mucosal and vascular changes in lung rejection and discuss their grading.

10041-12, Session 3

Idiopathic pulmonary fibrosis *(Invited Paper)*

Harold Collard, Univ. of California, San Francisco (United States)

No Abstract Available

10041-13, Session 3

Current diagnostic methods in pulmonary fibrosis, their limitations, and the need for improvement *(Invited Paper)*

Lida P. Hariri M.D., Massachusetts General Hospital (United States)

No Abstract Available

10041-14, Session 3

Optical coherence tomography as a diagnostic and therapeutic monitoring tool in pulmonary fibrosis *(Invited Paper)*

Melissa J. Suter, Massachusetts General Hospital (United States)

No Abstract Available

10041-15, Session 3

Technologies for optical elastography in the airways *(Invited Paper)*

David D. Sampson, The Univ. of Western Australia (Australia)

We shall review current technologies under the umbrella of optical elastography in the context of their suitability to characterise the mechanics of lungs, ranging from local compliance of the airway wall to the intrinsic micro-scale viscoelastic properties of constituent normal and pathological tissues. Key factors to be considered will include the length scale being probed, the capacity to be performed via a bronchoscope or endoscope, potential for integration with other functions, and requirement for contact with the tissue of interest. Emphasis will be placed on the technical capabilities of optical coherence elastography and optical coherence tomography.

10041-27, Session 3

AF-OCT for lung transplantation monitoring: a pilot study *(Invited Paper)*

Roland Nador M.D., BC Cancer Research Ctr. (Canada)

No Abstract Available

10041-16, Session 4

Value of polarisation-sensitive optical coherence tomography to evaluate the pleural layer both from chest wall tumor and from pleural reaction in rabbit model

Jung-Eun Park, Pukyong National Univ. (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Yeh-Chan Ahn, Ctr. for Marine-Integrated Biomedical Technology, Pukyong National Univ. (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Chulho Oak, Kosin Univ. (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Xin Zhou, Shuo Tang, The Univ. of British Columbia (Canada); Sung Won Kim, Hyoung Shin Lee, Kosin Univ. (Korea, Republic of) and Innovative Biomedical Technology Research Ctr. (Korea, Republic of); Eun-Kee Park, Kosin Univ. (Korea, Republic of)

Pleura is an important end-target organ after chronic exposure to environmental toxic materials such as fine articulate matters, asbestosis et ct. we evaluated pathological changes of pleura layer from animal model of localized chest wall tumor and localized pleural reaction after pleurodesis by polarisation-sensitive optical coherence tomography (PS-OCT) Using rabbit model, parietal pleural surfaces on chest walls with localized pleural reaction after pleurodesis using talc(group A) and with localized tumor(group B) were evaluated using PS-OCT. Normal chest wall(group C) was compared as control group. A transversal scanning time domain OCT system was used for two-dimensional cross-sectional imaging of parietal pleura to measure the thickness of pleura, birefringence of intercostal muscle in group A. we evaluate the characteristics of tumor invasion based on birefringence of tercostal muscle in gourp B. In group A,B, mucosal irregularity, elevation, thickening of the pleura were readily ualised by conventional OCT sytem, while the transitional area between pleura and muscle was unclear. However, PS-OCT showed clear discrimination between two layers using characteristic birefringence of muscle. The pleural reaction in talc group showed the significant thickening(average thickness : 95 ± 11.5?m), while the normal group showed normal to minimal thickening(average thickness : 14 ± 6.5?m). There was loss of birefringence of intercosal muscle according to the depth of tumor invasion in localized chest wall tumor group. PS-OCT allows tissue identification based on birefringence, providing additional information on pleural pathologies.

10041-17, Session 4

Orientation resolved optical coherence tomography for the assessment of airway smooth muscle mass pre and post bronchial thermoplasty in-vivo

Jasmin A. Holz, David C. Adams, Lida P. Hariri M.D., Chris Manley, Margit V. Szabari, Massachusetts General Hospital (United States); Sean Fleury, Seamus O'Shaughnessy, Jason Weiner, Boston Scientific Corp. (United States); Melissa J. Suter, Massachusetts General Hospital (United States)

In patients with severe asthma the airway smooth muscle (ASM) mass is increased and hypercontractile leading to an increase bronchoconstriction and asthma exacerbation. Bronchial Thermoplasty (BT) is a new therapeutic modality, which aims to decrease the ASM mass through thermal ablation thereby decreasing the potential for ASM driven bronchoconstriction and ultimately resulting in a reduction in asthma exacerbations and improved lung function. We have developed a new method, termed orientation resolved optical coherence tomography (OR-OCT), that allows us to differentiate ASM from surrounding mucosal tissues. In this study we evaluate the potential of OR-OCT to assess the ASM mass pre and post BT in-vivo. In a canine (n=8) preclinical model, imaging was performed during bronchoscopy using the in-house developed endobronchial OR-OCT system and catheters. We imaged selected airway segments pre and post BT in one side of the lung and on the other side as control. The treated and control segments were followed over time, imaged at day 21 and 42 post BT. At the end point, methacholine (Mch) was topically administered to 5 treated and 5 control segments per canine (n=4) to induce airway constriction. OR-OCT was performed pre and post Mch administration. Following sacrifice, the treated and control segments were histologically examined and matched with the OR-OCT data and analyzed. We will present our results from this experiment to assess the ASM mass pre and post BT using OR-OCT.

10041-18, Session 4

Polarization-sensitive optical coherence tomography (PS-OCT) imaging of systemic sclerosis

Margit V. Szabari, David C. Adams, Lida P. Hariri, David Lagares, Andrew M. Tager, Melissa J. Suter, Massachusetts General Hospital (United States)

Systemic Sclerosis is a chronic autoimmune disease of the connective tissue, which is characterized by increased collagen production, also known as fibrosis. It can affect various organs, such as the skin, the lungs, the digestive system and the kidney. Currently, there is no imaging modality which can visualize microscopic collagen deposition in vivo to help physicians diagnose and treat this condition better. Our overall goal is to use Polarization-Sensitive Optical Coherence Tomography (PS-OCT) to evaluate pulmonary fibrosis. We hypothesize that birefringence increases in tissues containing highly organized linear collagen fibers, which can be detected with PS-OCT. For validating this approach, in this conducting study, our aim was to test whether PS-OCT can detect and monitor the extent of fibrosis that develops and progresses in a mouse model of skin fibrosis.

Wild type mice were anesthetized, shaved and subcutaneously injected with Bleomycin (Treated group) or with saline (Control group) in the dorsal area for 28 days. Seven, 14, 21, and 28 days after the first injection, the skin of the mouse was imaged. The PS-OCT imaging system utilized a 68 kHz swept-source laser centered at 1300 nm and it was configured with an upright benchtop scanner. Following euthanasia of the mice, the imaged areas were harvested for further histological assessment.

Markedly increased birefringence was detected in the Treated group 28 days after the first injection compare to the Control group. Thus PS-OCT was able to provide valuable insight into the collagen deposition of connective tissue of the skin in fibrotic condition.

10041-19, Session 4

In-vitro investigation on the interaction of thulium-laser irradiation with bronchial stents

Ronald Sroka, Laser-Forschungslabor (Germany); Johannes Frank, Frank Reichenberger, J. Behr, Wolfgang Gesierich, Asklepios Kliniken München-Gauting (Germany)

Granulation and tumor regrowth in the area of bronchi stent implants may result in restenosis. It had been shown that by means of Thulium-Fibre-Laser (TFL) a controlled ablation and reduction of the tissue within the stent could be performed. When using Nd:YAG irradiation there is risk for explosive flames, burns of fibre and stent, ruptures of stent meshes as well as perforation of stent and cover. Therefore it was the aim to investigate the safety margin when using TFL.

Four different types of clinical used stents (with/without cover) were fixed to pig trachea tissue. Irradiation was performed by fibre assisted TFL-1940nm-laser irradiation while laser power, light application duration and distance, as well as oxygen percentage and contamination were varied.

In case of Nitinol-stents rupture were observed at power levels ≥7W or distances of <5mm, oxygen conc. of 40% result in increased flame appearance. Polyurethan-covers were ruptured at each variable, flame appeared at 5W. Silicon-stents were destroyed at power levels of about 5W and distances of <5mm and additionally 30%-oxygen or contamination either by blood or soot result in increased appearance of burns and flames.

Based upon these observations in clinical TFL-irradiation the distance should ≥5 mm and the power level should be ≤6W. Furthermore the oxygen conc. should not exceed 30% and short term continuous irradiation of less than 15s exposition should be considered. In case of Silicon-stents light application on contaminated area should be avoided.

10041-20, Session 4

Towards in situ bacterial detection in human alveolar lung tissue using bacterial probes and fluorescence lifetime imaging microscopy (FLIM)

Tushar R. Choudhary, Mark Bradley, The Univ. of Edinburgh (United Kingdom); Rory R. Duncan, Heriot-Watt Univ. (United Kingdom); Kevin Dhaliwal, The Univ. of Edinburgh (United Kingdom)

Antibiotic resistance is a serious global concern. One way to tackle this problem is to develop new and sensitive approaches to diagnose bacterial infections and prevent unnecessary antibiotic use. With recent developments in optical molecular imaging, we are one step closer to in situ rapid detection of bacterial infections. We present here bespoke fluorescent probes for bacterial detection in ex vivo human lung tissue using fluorescence lifetime imaging microscopy (FLIM). Two in-house synthesised bespoke probes were used in this study to detect and differentiate between Gram positive and Gram negative bacterial strain using their fluorescence lifetime in the ex vivo human lung tissue. The average fluorescence lifetime of Gram positive probe (n=12) was 2.40 ± 0.25 ns and Gram negative (n=12) was 6.73 ± 0.49 ns. The human lung tissue (n=12) average fluorescence lifetime value was found to be 3.43 ± 0.19 ns. Furthermore we were also able to distinguish between dead or alive bacteria in ex vivo lung tissue based on difference in their lifetime. We have developped Fibre-FLIM methods to enable clinical translation within the Proteus Project (www.proteus.ac.uk).

10041-21, Session 4

Fluorescent lifetime and multi-colour microendoscopy for intensive care unit diagnostic application

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We present a dual colour laser scanning endoscope capable of point-of-care fluorescent lifetime microendoscopy at one frame per second (fps). The scanning system is built as a standard confocal microscope that employs non-resonant galvos coupled to a coherent imaging fibre with ~30,000 cores and less than 1.4 mm packaged outer diameter enabling fast multicolour FLIM through an endoscope. When not used in FLIM mode the laser-scanning endoscope (LSE) achieves a speed of 10 fps by employing a spiral scanning pattern. Lifetime imaging is achieved by using a focal–plane array of 32 ? 32 single photon avalanche photodiode detectors (SPADs) that provides accurate photon arrival time. A 485 nm and a 635 nm lasers are pulsed at same repetition rate with a delay sufficient to separate two fluorescent decays in time.

To create lifetime data the SPAD array is used as a single–point detector but all the detectors contribute to the build-up of a histogram per scanned image elements, thus reducing impact from SPAD dead time and significantly increasing photon collection efficiency. A centre-of-mass algorithm with background subtraction is then used to determine the lifetime of each scanned point.

We show labelled bacteria differentiation in ex vivo samples of human lung against strong lung autofluorescence opening the road for clinical studies of a potentially lifesaving molecular imaging techniques for intensive care units.

10041-22, Session 4

Functional optical imaging of tracheal health

Daniel A. Gil, Joe T. Sharick, Vanderbilt Univ. (United States); Ute A. Gamm, Michael A. Choma, Yale Univ. (United States); Melissa C. Skala, Morgridge Institute for Research (United States) and Univ. of Wisconsin–Madison (United States)

The health of the tracheal mucosa is an important, but poorly understood, aspect of critical care medicine. Many critical care patients are mechanically ventilated through an endotracheal tube that can cause local inflammation and blunt damage to the ciliated epithelial cells lining the trachea. These cilia clear mucus and infectious agents from the respiratory tract, so impaired ciliary function may lead to increased susceptibility to respiratory infection. Therefore, a minimally-invasive method to monitor mucosal health and ciliary function in intubated patients would be valuable to critical care medicine. Optical metabolic imaging (OMI) can quantitatively assess the metabolic state of cells by measuring the fluorescence intensities of endogenous metabolic co-enzymes NAD(P)H and FAD. OMI is especially attractive for assessing tracheal health because OMI is label-free, and ciliary function is tightly linked to the levels of NAD(P)H and FAD. In this study, we apply widefield OMI to ex vivo mouse tracheae (n=6), and demonstrate that the optical redox ratio (fluorescence intensity of NAD(P)H divided by the intensity of FAD) is sensitive to changes in the cellular metabolism of the tracheal mucosa. We observed a 46% increase in the redox ratio 20 minutes after treatment with 10mM of sodium cyanide ($p<0.001$, 95% CI [40%, 52%]), an inhibitor of oxidative cellular respiration. In addition to being a proof-of-concept demonstration, Pseudomonas aeruginosa, an important cause of morbidity and mortality in CF patients and in the ICU, produces hydrogen cyanide. Our results support the development of minimally-invasive fiber-optic probes for in vivo monitoring of tracheal health.

10042-1, Session 1

Using molecular imaging to assess the delivery and infection of protease activated virus in animal model of myocardial infarction

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Cardiovascular diseases remain the greatest cause of death in the US and gene therapy has the potential to be an effective therapy. In this study, we demonstrated MMP-9 based protease-activatable viruses (PAVs) for selective infection of myocardial infarct (MI) that is associated with active MMP-9 expression. To test the specificity of PAVs, we used expression of a far-red fluorescence protein (iRFP) delivered by the PAV together with a dual PET/NIRF imaging agent specific for active MMP-9 activity at the site of MI in a murine model. Fluorescence imaging devices employed a highly-sensitive intensified camera, excitation light sources, and filtration schemes based upon the spectra of iRFP and the NIRF agent. One to two days after ligation of the left anterior descending artery, the PAVs or WT AAV9 virus (5x10^10 particles) encoding for iRFP and radiolabeled MMP-9 imaging agent (3 nmol) were injected intravenously (i.v.). PET imaging showed MMP activity was associated with adverse tissue remodeling at the site of the MI. One week after, animals were again injected i.v. with the MMP-9 agent (3 nmol) and 18-24 h later, the animals were euthanized and the hearts were harvested, sliced, and imaged for congruent iRFP transgene expression and NIRF signals associated with MMP-9 tissue activity. The margins of iRFP and NIRF contrasted tissues were quantified with Standard International units of mW/cm^2/sr. The sensitivity, specificity, and accuracy of PAV and WT targeting to sites of MI was determined. The PAVs demonstrated significantly higher delivery performance than that of the WT AAV9 virus.

10042-2, Session 1

Mapping the human atria with optical coherence tomography

Theresa H. Lye, Yu Gan, Christine P. Hendon, Columbia Univ. (United States)

Atrial structure plays an important role in the mechanisms of atrial disease. However, detailed imaging of human atria remains limited due to many imaging modalities lacking sufficient resolution. We propose the use of optical coherence tomography (OCT), which has micrometer resolution and millimeter-scale imaging depth well-suited for the atria, combined with image stitching algorithms, to develop large, detailed atria image maps.

Human atria samples (n=4) were obtained under approved protocols from the National Disease Research Interchange (NDRI). One right atria sample was imaged using an ultrahigh-resolution spectral domain OCT system, with 5.52 and 2.72 ?m lateral and axial resolution in air, respectively, and 1.78 mm imaging depth. Three left atria samples were imaged using the spectral domain OCT system, Telesto I (Thorlabs GmbH, Germany) with 15 and 6.5 ?m lateral and axial resolution in air, respectively, and 2.5 mm imaging depth. Overlapping image volumes were obtained from areas of the left and right atria and the pulmonary veins. The largest side length of a continuous imaged region was approximately 50 mm. Comparing with Trichrome histology, regions of collagen, adipose, and myocardium could be identified within the OCT images. Automated algorithms were applied for preliminary stitching of regions with side dimensions up to 20 mm. Next steps include stitching of the entire dataset and imaging of larger regions, as well as automated classification of tissue types within the stitched volumes. In the future, large OCT volumes could be registered with MRI to incorporate detailed tissue structure with overall 3D anatomy.

10042-3, Session 1

In vivo imaging of myocardial vasculature of a beating mouse heart using optical coherence tomography angiography

Inho Shin, KAIST (Korea, Republic of); Sun-Joo Jang M.D., Massachusetts General Hospital (United States); Hyun-Sang Park, Wang-Yuhl Oh, KAIST (Korea, Republic of)

Optical coherence tomography angiography (OCTA) is an emerging OCT-based technology that visualizes vasculatures of biological tissues without exogenous contrast agents. The OCTA has been widely used in many clinical and fundamental research areas such as ophthalmology, neurology, and various studies using small animal models. However, imaging blood vessel networks of the mouse heart in vivo is challenging due to the motion of the heart beat and limited temporal resolution of the OCT system. We demonstrate a swept-source OCT system (repetition rate of 220 kHz) for imaging the myocardial vasculature. The electrocardiogram (ECG) gated and the retrospective gated beam scanning methods were implemented to reduce the bulk cardiac motion. A custom-built motion stabilizer was also utilized to suppress the cardiac motion during imaging. The ECG gated beam scanning was realized that the galvanometric scanner operated in a proper time window which was expected to have the minimum cardiac motion according to the ECG signal. The retrospective gated scanning scheme performed multiple data acquisition at the same position and selected data that were minimally affected by the cardiac motion. The motion stabilization frame suppressed the cardiac motion and also provided the imaging window of the mouse heart. Following anesthesia mice were intubated and ventilated during imaging. This study would enable to provide important information in the study of ischemic cardiovascular diseases in vivo such as myocardial infarction.

10042-4, Session 1

Non-contact arrhythmia assessment in natural settings: a step toward preventive cardiac care

Robert Amelard, Univ. of Waterloo (Canada); Richard L. Hughson, Schlegel-UW Research Institute for Aging (Canada); David A. Clausi, Alexander Wong, Univ. of Waterloo (Canada)

Cardiovascular disease is a major contributor to US morbidity. Taking preventive action can greatly reduce or eliminate the impact on quality of life. However, many issues often go undetected until the patient presents a physical symptom. Non-intrusive continuous cardiovascular monitoring systems may make detecting and monitoring abnormalities earlier feasible. One candidate system is photoplethysmographic imaging (PPGI), which is able to assess arterial blood pulse characteristics in one or multiple individuals remotely from a distance. In this case study, we showed that PPGI can be used to detect cardiac arrhythmia that would otherwise require contact-based monitoring techniques. Using a novel system, coded hemodynamic imaging (CHI), strong temporal blood pulse waveform signals were extracted at a distance of 1.5 m from the participant using 850-1000 nm diffuse illumination for deep tissue penetration. Data were recorded at a sampling rate of 60 Hz, providing a temporal resolution of 17

ms. The strong fidelity of the signal allowed for both temporal and spectral assessment of abnormal blood pulse waveforms, ultimately to detect the onset of abnormal cardiac events. Data from a participant with arrhythmia was analyzed and compared against normal blood pulse waveform data to validate CHI's ability to assess cardiac arrhythmia. Results indicate that CHI can be used as a non-intrusive continuous cardiac monitoring system.

10042-5, Session 1

Evaluation of structural remodeling of the atria with optical coherence tomography in a chronic rat model of myocardial infarction

Melissa M. Eberle, Stephanie Thorn, Lawerence Young, Daniel Pfau, Yale Univ. (United States); Jeffrey Madwed, Kersten Small, Michael Kilmas, Merck Research Labs. (United States); Michael A. Choma, Yale School of Medicine (United States); Albert J. Sinusas, Yale Univ. (United States)

Atrial fibrillation (AF) occurs following myocardial infarction (MI) and is associated with left ventricular dysfunction, which promotes the development of atrial remodeling and permanent atrial fibrosis. The purpose of this study was determining the effects of MI on left atrial (LA) remodeling with and without therapy with an angiotensin converting enzyme inhibition (ACEi) utilizing optical coherence tomography (OCT). As the composition of the myocardial tissue changes during LA remodeling the optical attenuation of the light will also change providing a metric to quantify the structural remodeling process. Lewis rats (240-275 g) underwent either surgical ligation of left coronary artery creating chronic MI, or SHAM surgery. 13 weeks post-surgery, ex vivo OCT imaging was performed of the LA appendage. Depth-resolved, attenuation coefficient volumes were calculated and the resulting atrial wall attenuation values were analyzed for four experimental groups: SHAM, SHAM with ACEi, MI no ACEi, and MI with ACEi. Quantification of tissue attenuation was performed and shown to significantly increase with MI in association with increases in collagen as verified with corresponding histological sectioning. Fractal analysis of the LA wall trabeculation patterns, 100 μm below the surface, was performed to quantify wall thickening associated with LA remodeling. A significant increase in fractal dimension was determined post MI compared to SHAM corresponding to a loss of the trabeculation pattern and wall thickening. The results from this study demonstrate OCT as an imaging technique capable of investigate LA remodeling with high resolution and label-free optical contrast processing.

10042-6, Session 1

Characterization of ventricular endomyocardial tissue using optical coherence tomography

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With a penetration depth up to 2 mm, similar to the size of most biopsy samples?optical coherence tomography (OCT) has the ability to characterize the endomycoardial biopsy (EMB) site without physical excision and has great potential to guide EMB. Our objective is to characterize ventricular myocardial samples to facilitate the diagnosis of endomyocardial disease.

We conducted imaging on three types of ventricular samples, including human hearts (n = 15) through national disease research interchange tissue program within 48 h of donor death, endomyocardial biopsy samples from patients being assessed for transplant rejection (n = 10), and surgical samples (n = 6). The first dataset includes samples with myocardial infarction while the second and the third samples include samples with amyloid. Trichrome histology and pathology were processed after imaging.

Initial automated analysis was conducted within samples from the first dataset. We performed layer thickness study and text feature analysis, including homogeneity and entropy on tissue types of scar, normal myocardium, normal endocardium, and adipose tissue. OCT B-scans were segmented into multiple layers based on a cost function consisting of intensity, gradient, and curve smoothness.

We found that the thickness of endocardium (0.121 mm ± 0.044 mm) in scar was significantly larger than that (0.068 mm ± 0.019 mm) in normal tissue. With higher entropy and lower homogeneity, adipose tissue appeared in one ventricular septum, indicating a high risk of arrhythmogenic cardiomyopathy. Our next step is to characterize the amyloid type in larger dataset within biopsy using spectroscopic OCT.

10042-7, Session 2

Fully integrated optical coherence tomography, ultrasound, and indocyanine green based fluorescence tri-modality system for intravascular imaging

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The rupture of atherosclerotic plaques is the leading cause of acute coronary events, so accurate assessment of plaque is critical. A large lipid pool, thin fibrous cap, and inflammatory reaction are the crucial characteristics for identifying vulnerable plaques. In our study, a tri-modality imaging system for intravascular imaging was designed and implemented. The tri-modality imaging system with a 1-mm probe diameter is able to simultaneously acquire optical coherence tomography (OCT), intravascular ultrasound (IVUS), and fluorescence imaging. Moreover, for fluorescence imaging, we used the FDA-approved indocyanine green (ICG) dye as the contrast agent to target lipid-loaded macrophages. Firstly, IVUS is used as the first step for identifying plaque since IVUS enables the visualization of the layered structures of the artery wall. Due to low soft-tissue contrast, IVUS only provides initial identification of the lipid plaque. Then OCT is used for differentiating fibrosis and lipid pool based on its relatively higher soft tissue contrast and high sensitivity/specificity. Last, fluorescence imaging is used for identifying inflammatory reaction to further confirm whether the plaque is vulnerable or not. Ex vivo experiment of a male New Zealand white rabbit aorta was performed to validate the performance of our tri-modality system. H&E histology results of the rabbit aorta were also presented to check assessment accuracy. The miniature tri-modality probe, together with the use of ICG dye suggest that the system is of great potential for providing a more accurate assessment of vulnerable plaques in clinical applications.

10042-8, Session 2

Contact optical rotary junction for multi-modality optical coherence tomography and near-infrared fluorescence/ autofluorescence imaging

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Fiber based multimodality endoscopic imaging techniques have been widely investigated for diagnosis of the disease in coronary artery, lung, gastrointestinal tract, etc. For example in cardiology, intravascular optical coherence tomography (OCT) combined with near-infrared fluorescence/autofluorescence (OCT-NIRF/OCT-NIRAF) can provide the high-resolution microstructure of the coronary artery wall, as well as co-localized molecular information for more precise diagnosis of the plaques.

Typically, an intravascular multimodality catheter is made from a double cladding fiber (DCF), where the OCT signal is guided through the single mode core and fluorescence emission is transmitted through the multimode inner cladding in order to improve collection efficiency. An optical rotary junction (RJ) enables the transmission of the light between static imaging system and rotating catheter, has the challenges of optimizing the core-to-core, the cladding-to-cladding light transmission efficiency and minimizing the chromatic aberration. These optical operations need to be performed efficiently while at the same time achieving compactness and mechanical stability at the high rotating speeds that are required by the clinical application.

Here, we present a compact RJ with an optical contact design. The static and rotating fibers are in near contact with an intervening index matching gel to obtain maximum transmission efficiency and reduce the back reflections from fiber-air interfaces. The OCT signal from the DCF core was coupled into the OCT system from the single mode port of a DCF fiber coupler and the multimode port was connected to a remote fluorescence spectroscopy detection system. The insertion loss with angle variation was measured and analyzed. The proposed RJ was validated by ex vivo OCT-NIRF and OCT-NIRAF imaging of coronary arteries at 100 frame/s. Results show that stable and high signal to noise ratio OCT and fluorescence data can be achieved with the device.

10042-9, Session 2

Detection and characterization of atherosclerotic plaques by Raman probe spectroscopy and optical coherence tomography

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Visualization and characterization of inner arterial plaque depositions is of vital diagnostic interest. Established intravascular imaging techniques provide valuable morphological information, but cannot deliver information about the chemical composition of individual plaques. Probe based Raman spectroscopy offers the possibility for a biochemical characterization of atherosclerotic plaque formations during an intravascular intervention. From post mortem studies it is well known that the severity of a plaque and its stability are strongly correlated with its biochemical composition. Especially the identification of vulnerable plaques remains one of the most important and challenging aspects in cardiology. Thus, specific information about the composition of a plaque would greatly improve the risk assessment and management. Furthermore, knowledge about the composition can offer new therapeutic and medication strategies. Plaque calcifications as well as major lipid components such as cholesterol, cholesterol esters and triglycerides can be spectroscopically easily differentiated. Intravascular optical coherence tomography (OCT) is currently a prominent catheter based imaging technique for the localization and visualization of atherosclerotic plaque depositions. The high resolution of OCT with 10 to 15 μm allows for very detailed characterization of morphological features such as different plaque formations, thin fibrous caps and accurate measurements of lesion lengths. In combination with OCT imaging the obtained spectral information can provide substantial information supporting on on-site diagnosis of various plaque types and therefor an improved risk assessment. The potential and feasibility of combining OCT with Raman spectroscopy is demonstrated on excised plaque samples, as well as under in vivo conditions.

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10042-10, Session 2

A dual modality imaging system integrating optical frequency domain imaging (OFDI) and intravascular ultrasound imaging (IVUS) for intravascular diagnosis

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Recent studies have suggested the diagnostic value of combining both optical and acoustic imaging inside vessels. Optical frequency domain imaging (OFDI) has successfully resolved various intravascular structures at a resolution of <20 microns. However, its imaging depth is limited to 1~2mm due to tissue scattering. Intravascular ultrasound imaging (IVUS) can indeed image through entire vessel walls, enabling in-vivo plaque burden estimation. Yet, its current resolution is about several hundred microns, preventing it from visualizing microstructures in the vessels. In light of their complementary traits, the synergistic combination of OFDI and IVUS might provide a more comprehensive characterization of intravascular pathology. For example, thin-cap fibroatheroma (TCFA), a predominant type of vulnerable plaque with high risk of atherosclerotic complication, could potentially be detected by such a dual modality system, where IVUS images showing plaque burden and OFDI images disclosing cap thickness are acquired simultaneously and co-registered automatically. Aiming to the clinical translation of this approach, we present our next-generation combined IVUS/OFDI system. It includes an imaging console offering concurrent OFDI and IVUS axial scans at a rate of over 40kHz. Clinical grade catheters (2.6Fr) containing optical ball lens and ultrasonic transducers were designed and fabricated. In this updated system, a new leak-proof rotary joint was developed and tested under different flushing protocols, allowing for reliable operation in the cardiac catheterization lab. Improved data processing and user interfaces provide enhanced visualization and easier control. Animal and human in-vivo imaging studies are ongoing and expected to demonstrate the unique benefits of this hybrid system.

10042-11, Session 2

Fluorescence lifetime intravascular ultrasound (FL-IVUS) and the quest to discriminate between early and advanced lipid cores in atherosclerosis

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FL-IVUS combines intravascular ultrasound with fluorescence lifetime imaging to obtain morphologic and biochemical details from the arterial wall. Ultrasound measurements alone provide morphologic information (plaque burden, remodeling index and presence of calcium). Fluorescence lifetime can determine the presence of a thick fibrous cap, macrophage infiltration, and lipid cores beneath thin fibrous caps. These details are important to assess plaque vulnerability. In this study, we focused on the ability of FL-IVUS to differentiate between early and advanced lipid cores—advanced cores are vulnerable to rupture. We imaged N=12 ex vivo human coronary arteries and performed hematoxylin and eosin, Movat's pentachrome and CD68 immunohistochemistry at 500 micron intervals throughout the length of the vessels. We found only N=1 thin-capped fibroatheroma (TCFA) with an advanced necrotic core and N=7 cases of foam cell infiltration, early lipid cores or deep necrotic cores. IVUS was able to observe the increased plaque burden and calcification of the advanced and deep necrotic cores, but could not identify early lipid cores, foam cell infiltration or discriminate between deep necrotic cores and TCFA. The addition of FLIm to IVUS allowed the TCFA to be discriminated from early lipid accumulation, particularly at 542±50 nm (355 nm pulsed excitation): 7.6 ± 0.5 ns compared to 6.6 ± 0.4 ns, respectively ($P<0.001$ by ANOVA analysis). These differences need to be validated in a larger cohort, but exist due to specific lipid content in the necrotic core as well as increased extracellular matrix in early lesions.

10042-12, Session 3

In-Situ laser fenestration of endovascular stent-graft in abdonimal aortic aneurysm repair (EVAR)

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Endovascular abdominal aortic aneurysms repair (EVAR) involves the minimally invasive implantation of a stent-graft within the aorta to exclude the aneurysm from the circulation thus preventing its rupture.

The feasibility of such operation is highly dependent on the aorta morphology and in general the presence of one/both renal arteries emerging from the aneurysm is the absolute limit for the implantation of a standard stent-graft. Consequently, classical intervention methods involve the implantation of a custom-made graft with fenestrations, leading to extremely complicated surgeries with high risks for the patient and high costs.

More recent techniques introduced the use of standard grafts (i.e. without fenestrations) with mechanical in-situ fenestration, but this procedure is limited principally by the brittleness and low stability of the environment, in addition to the difficult of guidance of the endovascular tools due to the temporarily block of blood flow.

In this work we propose an innovative EVAR strategy which involves in-situ fenestration with a fiber guided laser tool, controlled via an electromagnetic navigation system.

The fiber is sensorized to be tracked via the driving system and, using a 3D model of the patient anatomy, the surgeon can drive it on the aneurysm, where the stent has been released, to realize the proper fenestration(s).

The realization of the catheter laser tool will be presented, togheter with preliminary fenestration tests on graft-materials, including the effect of blood and tissues presence.

10042-13, Session 3

In vitro investigation of photoplethysmography in the detection of platelet activation

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Evidence continues to build on the role of platelets as initial contributors in the development of atherosclerotic lesions. Nevertheless, routine clinical tests do not include haemorheological assessments for cardiovascular patients. This study investigates the sensitivity of the optical technique, Photoplethysmography (PPG), for the detection of changes in blood characteristics in a controlled setup during a controlled thromboplastin activation.

An in vitro setup that mimics the human circulation allowing control of the haemodynamic forces on the wall/fluid interface while circulating donor equine blood was developed. Equine blood is known to be suitable for comparison to human blood due to the size of the cells and their ability to aggregate. Custom-made PPG sensors, Ultrasound flow Doppler and pressure sensors were fitted on the model enabling continuous real-time measurements. A Matlab script was also developed for analysing the acquired signals. PPG signals showed significant responses to changes in platelet activation, specifically a significant ($p<0.001$) drop in PPG amplitudes and changes in signal morphology were observed. To our knowledge, the effect of platelet activation on the PPG has not been investigated before. This in vitro study has demonstrated that the PPG is highly sensitive to changes in haemorheology due to clot formations. These preliminary findings provide the first positive steps suggesting that the technique of Photoplethysmography might be able to contribute to the non-invasive monitoring of markers relating to cardiovascular diseases such as the early stage of blood clot formations and viscosity changes. More rigorous studies are needed in order to investigate further the capability of the PPG in detecting such blood properties.

10042-14, Session 3

An optical system for high-throughput screening of cardiac electrophysiology for human cardiomyocytes

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Cardiomyocytes derived from human induced pluripotent stem cells (hiPS-HCM) have the potential to provide individualized therapies for patients and to test drug candidates for cardiac toxicity. In order for hiPS-CM to be useful for such applications, there is a need for high-throughput technology to rapidly assess cardiac electrophysiology parameters. Here, we designed and tested a fully contactless optical mapping (OM) and optical pacing (OP) system capable of imaging and point stimulation of hiPS-CM in small wells. OM allowed us to characterize cardiac electrophysiological parameters (conduction velocity, action potential duration, etc.) using voltage-sensitive dyes with high temporal and spatial resolution over the entire well. To improve OM signal-to-noise ratio, we tested a new voltage-sensitive dye (Fluovolt) for accuracy and phototoxicity. Stimulation is essential because most electrophysiological parameters are rate dependent; however,

traditional methods utilizing electrical stimulation is difficult in small wells. To overcome this limitation, we utilized OP (? = 1464 nm) to precisely control heart rate with spatial precision without the addition of exogenous agents. We optimized OP parameters (e.g., well size, pulse width, spot size) to achieve robust pacing and minimize the threshold radiant exposure. Finally, we tested system sensitivity using Flecainide, a drug with well described action on multiple electrophysiological properties.

10042-15, Session 3

Quantitative comparison between radial and cylindrically diffusing fibers for photothermal treatment of varicose veins disease

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For last two decades, endovenous laser therapy (EVLT) is one of the most widely accepted surgical options for treating incompetent great and small saphenous veins. However, due to excessive heating during EVLT, the major complications include pain and burning that often increase the risk of dermatitis disease. The aim of the current study was to quantitatively compare commercially-available radial fibers with newly-developed diffusing applicators for 1470 nm-EVLA in terms of temperature elevation and vein deformation. Rabbit veins were used as an ex vivo model for EVLA. A 5-W 1470 nm laser system in conjunction with the radial and diffusing fibers was employed to thermally coagulate the venous tissue. A goniometric measurement validated uniform and isotropic distribution of laser light in polar and longitudinal directions (i.e., normalized intensity = 0.84±0.08). The diffusing applicator induced a 20 % lower maximum temperature than the radial fiber did (maximum temperature = 79.2 °C for radial vs. 63.3 °C for diffusing). Due to higher irradiance, the radial fiber was associated with a transient temperature change of 5.9 °C/s, which was 1.5-fold faster than the diffusing applicator (i.e., 2.4 °C/s). However, the degree of cross-sectional area reduction in the veins was almost comparable for both the fibers (i.e., 53% for radial vs. 48% for diffusing). Due to longer irradiation length, the diffusing applicator demonstrated wider treatment coverage and less fiber speed-dependent. On account of easy pullback technique and uniform thermal effect, the proposed cylindrically diffusing applicator can be a feasible optical device to effectively treat varicose veins. Further in vivo studies will be performed to identify the complete removal of the vein disease and healing response of the venous tissue.

10042-16, Session 4

Attenuation and backscattering based tissue characterization in intravascular optical coherence tomography

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Intravascular optical coherence tomography (IVOCT) is a new intravascular imaging modality which enables arterial structures to be visualized at a microstructure level. The identification of the different structures in the arterial wall is currently performed visually based upon relative light intensities. This is complicated since there are many factors influencing the absolute intensities in the IVOCT image, including the catheter position inside the artery and vendor of the catheter.

In this study we demonstrate how light attenuation and backscattering values can be computed and used as better characterizing features for different types of atherosclerotic plaque such as fibro-atheroma, lipid-pools and calcified areas.

To validate the method, different plaque components are segmented in multiple ex-vivo IVOCT pullback runs using matching expert annotated histology-data. The optical attenuation, backscattering and light intensity features of the segmented regions are then automatically extracted and analyzed for their entropy with regards to tissue characterization.

The results of the validation analysis show that the computed attenuation and backscattering measurements are in agreement with those published in literature and that especially attenuation is a more robust feature than light intensity for tissue characterization. As a practical application we show how attenuation and backscattering can be used to quickly determine the presence of lipid or calcified plaques which are important factors for treatment planning. Based on these findings we intend to develop a fully automatic tissue characterization method for IVOCT.

10042-17, Session 4

Bioresorbable vascular scaffold assessment by combination of the ECG-triggered high-speed single cardiac cycle OCT system and the OCT/NIRF Dual Modal system.

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Bioresorbable vascular scaffold (BVS) may have risk of disruption due to its resorbing characteristic that potentially leads mechanical weakening. Fracture of the stents may serve as a substrate for thrombosis and inflammation. Current 2D OCT-based BVS studies may not provide sufficient information to fully understand this process. Large longitudinal pitch and heart motion induced image distortion of current low frame rate OCT possibly miss small fractures. Additionally, structural information alone provided by OCT has limitation to access inflammatory processes occurring in the vessel wall.

We present early experience of assessment of BVS fracture using a combination of two intracoronary imaging systems. ECG-triggered high-speed single cardiac cycle OCT provided detailed structural information of the BVS. Fully integrated OCT/near-infrared-fluorescence (NIRF) dual modal system identified relationship between inflammation and fracture.

BVS was implanted within the coronary artery of Yorkshire pigs while they were anesthetized and externally ventilated. Balloons were excessively expanded to make fracture on the BVS. We sequentially performed ECG-triggered high-speed OCT imaging and OCT/NIRF imaging on the affected area.

In ECG-triggered OCT imaging process, all regions of interest were automatically imaged within a portion of a heart beat with minimum motion at a frame rate of 500 fps. For OCT/NIRF imaging, indocyanine-green were used as exogenous contrast agents to detect stent fracture associated inflammation. Conventional speed OCT imaging was also performed as a comparison.

The high-speed ECG-triggered OCT effectively detected BVS fractures and the fluorescence signals acquired from the OCT/NIRF system matched well with the associated fracture sites.

10042-18, Session 4

Optical signatures of lipid droplets and cholesterol crystals in atheromatous plaques

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Polarization sensitive optical coherence tomography (PS-OCT) measures the polarization states of the light backscattered by tissue and provides measures of tissue birefringence and depolarization in addition to the backscatter intensity signal. The conventional structural signal identifies lipid-rich plaques as signal-poor regions with poorly delineated borders and a signal that is rapidly decaying along depth. In histopathology studies of cadaveric human hearts with PS-OCT, we found that the majority of these lesions exhibit substantial depolarization and efficiently scramble the polarization state of the input light. Atheromatous plaques consist primarily of macrophage cells that have ingested substantial amounts of lipids, stored as intracellular lipid droplets. Excessive concentration of cholesterol leads to the nucleation of small cholesterol crystals, which have received increased attention for their suggested role in physically piercing and weakening the fibrous cap as well as in inciting inflammation. Here, we demonstrate with measurements of artificial lipid emulsions and synthetic monohydrate cholesterol crystals that lipid droplets on their own cannot account for the observed signatures in the PS-OCT signal. In contrast, the birefringent and anisotropically scattering cholesterol crystals define a strongly depolarizing medium, reminiscent of the signal observed in lipid-rich plaques of human cadaver hearts. These results suggest that the depolarization observed in the PS-OCT signal is indicative of cholesterol crystals, and could offer novel perspectives for studying the contribution of this important component of atherosclerosis to the progression and complications of this disease.

10042-19, Session 4

Machine learning voxel-based coronary artery plaque classification from IVOCT images

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Intravascular Optical Coherence Tomography (IVOCT) imaging has the resolution, contrast, and penetration depth to characterize coronary artery plaques. Visual interpretation of a 500-image pullback is difficult even for experienced cardiologists, especially during a stressful interventional procedure. We created a method to automatically classify plaque tissues as fibrous, calcified, or lipid-rich. For this multi-class problem, we used one-versus-rest SVM classifiers for each of the three plaque types, with accommodation to exclude “other” voxels, and both physics-inspired and local texture features to classify voxels. Manually labeled clinical sub-volumes from 35 in-vivo pullback data sets (9485 image frames) constituted training and testing data. In addition, the trained algorithm was evaluated on independent ex-vivo image data accurately labeled using registered 3D microscopic cryo-imaging. Measured optical properties were in agreement with previous results reported in literature. Experiments on the clinical training data yielded 5-fold, voxel-wise accuracy of 87±9%, 96±5% and 97±3% for calcified, lipid-rich, and fibrotic tissues, respectively. Importantly, the algorithm correctly discriminated calcium and lipid, often a challenge to cardiologist, with <5% voxel error rates. Experiments on the independent validation data yielded 88% accuracy over all plaque types indicating generalizability. Since voxel-wise classification is noisy and presents greater resolution than clinically needed, we applied plurality voting within sectors of the independent dataset and obtained improved accuracy. Visualizations of plaque-labeled images allow one to rapidly review results and make informed treatment decisions.

10042-20, Session 4

Imaging birefringent crystals using polarization sensitive micro optical coherence tomography

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Background: Uric acid crystals have recently been identified as a possible therapeutic target for coronary artery disease. Being subcellular in size, it is difficult to identify these crystals in situ. Micro optical coherence tomography (Micro-OCT) allows one to image subcellular structures with 1–micron resolution. Even though Micro-OCT should be capable of resolving urate crystals, it’s difficult to differentiate these structures from other scattering particles within tissue. In this work we developed a novel polarization sensitive micro OCT (ps-Micro-OCT) system for identification of uric acid crystals.

Methods: A spectrometer based ps-Micro-OCT system was developed using a broadband light source. The broadband input light was divided into reference and sample signals using a beam splitter. The reference signal was further divided into two polarized signals with different polarization states. Reflected reference and sample signals were combined and sent to a spectrometer that recorded the interference signal.

Results: To test the performance of system, a mirror was used as sample and a quarter wave-plate was placed in the sample path. The measured quarter wave-plate angle values matched closely to actual angle values. Next we prepared uric acid crystals in our lab and imaged them using this system. We were able to image and identify these crystals based on polarization measurements.

Conclusion: In this work we imaged and identified uric acid crystals using a newly developed ps-Micro-OCT system. The proposed technique will enable imaging uric acid crystals in coronary artery.

10042-21, Session 5

Polarization-resolved SHG microscopy in cardiac hypertrophy study

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Cardiac hypertrophy, a process initiated by mechanical alterations, is hypothesized to cause long-term molecular-level alteration in the sarcomere lattice, which is the main force-generating component in the heart muscle. This molecular-level alteration is beyond the resolving capacity of common light microscopy. Second harmonic generation (SHG) microscopy has unique capability for visualizing ordered molecular structures in biological tissues without labeling. Combined with polarization imaging technique, SHG microscopy is able to extract structural details of myosin at the molecular level so as to reveal molecular-level alterations that occur during hypertrophy. The myosin filaments are believed to possess C6 symmetry; thus, the nonlinear polarization response relationship between generated second harmonic light I^2?and incident fundamental light I^? is determined by nonlinear coefficients, ?_15, ?_31 and ?_33. ?_31/?_15 is believed to be an indicator of the molecular symmetry of myosin filament,

while?_33/?_15represents the intramyosin orientation angle of the double helix. By changing the polarization of the incident light and evaluating the corresponding SHG signals, the molecular structure of the myosin, reflected by the ? coefficients, can be revealed. With this method, we studied the structural properties of heart tissues in different conditions, including those in normal, physiologically hypertrophic (heart tissue from postpartum female rats), and pathologically hypertrophic (heart tissue from transverse-aorta constricted rats) conditions. We found that ratios of ?_31/?_15 showed no significant difference between heart tissues from different conditions; their values were all close to 1, which demonstrated that Kleinman symmetry held for all conditions. Ratios of ?_33/?_15 from physiologically or pathologically hypertrophic heart tissues were raised and showed significant difference from those from normal heart tissues, which indicated that the intramyosin orientation angle of the double helix was altered when heart tissues hypertrophied. Polarization-resolved SHG microscopy permitted us to study heart tissues at the molecular level and may serve as a diagnostic tool for cardiac hypertrophy.

10042-22, Session 5

Towards optical spectroscopic anatomical mapping for lesion validation in cardiac tissue

Rajinder P. Singh-Moon, Mohammad Zaryab, Christine P. Hendon, Columbia Univ. (United States)

Electroanatomical mapping (EAM) is an invaluable tool for guiding cardiac radiofrequency ablation (RFA) therapy. The principle roles of EAM is the identification of candidate ablation sites by detecting regions of abnormal electrogram activity and lesion validation subsequent to RF energy delivery. However, incomplete lesions may present interim electrical inactivity similar to effective treatment in the acute setting, despite efforts to reveal them with pacing or drugs, such as adenosine. Studies report that the misidentification and recovery of such lesions is a leading cause of arrhythmia recurrence and repeat procedures. In previous work, we demonstrated spectroscopic characterization of cardiac tissues using a fiber optic-integrated RF ablation catheter. In this work, we introduce OSAM (optical spectroscopic anatomical mapping), the application of this spectroscopic technique to obtain 2-dimensional biodistribution maps. We demonstrate its diagnostic potential as an auxiliary method for lesion validation in treated swine preparations.

Endocardial lesion sets were created on fresh swine cardiac samples using a commercial RFA system. An optically-integrated catheter console fabricated in-house was used for measurement of tissue optical spectra between 600-1000nm. Three dimensional, Spatio-spectral datasets were generated by raster scanning of the optical catheter across the treated sample surface in the presence of whole blood. Tissue optical parameters were recovered at each spatial position using an inverse Monte Carlo method. OSAM biodistribution maps showed stark correspondence with gross examination of tetrazolium chloride stained tissue specimens. Specifically, we demonstrate the ability of OSAM to readily distinguish between shallow and deeper lesions, a limitation faced by current EAM techniques. These results showcase the OSAMs potential for lesion validation strategies for the treatment of cardiac arrhythmias.

10042-23, Session 5

Robust classification of contact orientation between tissue and spectroscopic RF catheter

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The quality of radiofrequency ablation (RFA) therapies for atrial fibrillation is dependent upon successful lesion formations. Notwithstanding improvements to these procedures, prior studies suggest that success rates, roughly 65% for one-time procedures, need improvement (Ganesan et al). Studies also favor using light-based catheters over conventional ones, as they grant the ability to accurately derive tissue properties such as lesion depth and charring from spectroscopic information (Demos et al). However, this spectroscopic information is heavily reliant on contact quality with the treatment area and the orientation of the catheter. Thus to improve assessments of tissue properties, this work utilizes machine learning to classify whether the catheter is indeed in proper contact with the tissue.

Six fresh swine hearts submerged in blood were experimented on using a commercial RFA catheter inserted into a fiber optic sheath. Tissue diffuse reflectance spectra (435nm–1145nm) was measured within both atriums and ventricles. Measurements of “full contact”, “1 millimeter off”, “2 millimeters off” and “pure blood” were collected. Multiple Bayesian classifiers were generated using Adaboost and fed into a K-means algorithm. The learning features included the area under the spectra and the slope between 800nm-1000nm, which decrease and increase respectively when the catheter is not in contact. Upon training on 100 unique spectra and testing on 600, 100% accuracy was attained in differentiating full perpendicular contact and no contact. Currently we applying the algorithm in vivo and evaluating at various contact angles. This allows invalid spectra to be filtered out when deriving tissue properties in the future.

10042-24, Session 5

Multivariate analysis of multispectral fluorescence lifetime imaging (FLIm) data: application to atherosclerotic plaque staging

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Multispectral fluorescence lifetime imaging (FLIm) is a fast, non-destructive, and non-invasive technique able to provide spectroscopic information of biological samples. It relies on the excitation of endogenous fluorophores using pulsed UV light, which provides spectral and temporal parametric maps. Spectral information alone is often not sufficient to unmix biological compounds characterized by wide emission bands; adding fluorescence decay parameters enables improved discrimination. While there is a well-established framework for the study of hyperspectral images, adding temporal informational creates additional challenges. In particular, the ability to extract accurate decay parameters for a given spectral band depends from the amount of signal collected in this band: for a given artery sample, variations of SNR between 15dB and 49dB across the field of view lead to a standard deviation of the recovered lifetime ranging between +/-0.32ns and +/-0.037ns, leading to varying noise properties across the field of view. Additionally, many unmixing methods rely on bilinear mathematical model assumptions that are not applicable to decay parameters. Lifetime-specific methods exist but do not include spectral information. In this work, we evaluate the suitability of a various types of clustering and classification techniques to multispectral FLIm data analysis and identify the best choices for (1) supervised classification for identification of clinically relevant tissue types identified by histology, and (2) unsupervised clustering for identification of chemical changes not yet correlated to histology changes. Clustering and classification methods are applied to the analysis of ex vivo human coronary data and the results discussed with respect to histological findings.

10042-25, Session 5

A preliminary study of fluorescence spectroscopy and wide-field UV autofluorescence imaging in atherosclerotic human aorta

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The aim of our study was to identify fluorescence excitation-emission pairs correlated with atherosclerotic pathology in ex-vivo human aorta. Wide-field images of atherosclerotic human aorta were captured using UV and visible excitation and emission wavelength pairs of several known fluorophores to view correspondence with gross pathologic features. Fluorescence spectroscopy and histology were performed on 21 aortic samples. A matrix of Pearson correlation coefficients were determined for the relationship between relevant histologic features and the intensity of emission for 427 wavelength pairs. A multiple linear regression analysis indicated that elastin (370/460 nm) and tryptophan (290/340 nm) fluorescence predicted 58% of the variance in intima thickness (R-squared = 0.588, F(2,18) = 12.8, p=.0003), and 48% of the variance in media thickness (R-squared = 0.483, F(2,18) = 8.42, p=.002), suggesting that endogenous fluorescence intensity at these wavelengths can be utilized for improved pathologic characterization of atherosclerotic plaques.

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10043-1, Session 1

Analysis of stromal alterations in ovarian cancers via wavelength dependent second harmonic generation microscopy and optical scattering *(Invited Paper)*

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Ovarian cancer remains the most deadly gynecological cancer with a poor aggregate survival rate. To improve upon this situation, we utilized collagen-specific Second Harmonic Generation (SHG) imaging microscopy and optical scattering measurements to probe structural differences in the extracellular matrix of normal stroma, benign tumors, endometrioid tumors, and low and high-grade serous (LGS and HGS) tumors. The SHG signatures of the emission directionality and conversion efficiency as well as the optical scattering are related to the organization of collagen on the sub-micron size. The wavelength dependence of these readouts adds additional characterization of the size and distribution of collagen fibrils/fibers relative to the interrogating wavelengths. We found strong wavelength dependent dependencies of these metrics that were different between the different tumors that are related to respective structural attributes in the collagen organization. These sub-resolution determinations are consistent with the dualistic classification of type I and II serous tumors. However, type I endometrioid tumors have strongly differing ECM architecture than the serous malignancies. Moreover, our analyses are further consistent with LGS and benign tumors having similar etiology. We identified optimal wavelengths for the SHG metrics as well as optical scattering measurements. The SHG metrics and optical scattering measurements were then used to form a linear discriminant model to classify the tissues, and we obtained high accuracy (~90%) between the tissue types. This delineation is superior to current clinical performance and has potential applicability in supplementing histological analysis, understanding the etiology, as well as development of an in vivo screening tool.

10043-2, Session 1

Mueller matrix colposcope for in-vivo cervical collagen imaging of the uterine-cervix

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Preterm birth presents a ubiquitous health issue worldwide affecting approximately10% of births and proving to be the number one cause of infant mortality and neurological disorders. To date, there is not an accurate, reliable diagnostic method for preterm birth. Collagen, the primary constituent of the cervix, provides the structural support and mechanical strength to maintain cervical closure, through specific organization, during fetal gestation. As pregnancy progresses, the disorganization of the cervical collagen occurs to allow eventual cervical pliability so the baby can be birthed through the cervical opening. This disorganization of collagen affects the mechanical properties of the cervix and, if the changes occur prematurely, may be a significant factor leading to preterm birth. The organization of collagen can be analyzed through the use of Mueller Matrix Polarimetric imaging of the characteristic birefringence of collagen. In this research, we have built a full Mueller Matrix Polarimeter attachment to a standard colposcope and have imaged human cervixes, in-vivo. Using Mueller Matrix decomposition, we have analyzed these polarimetric images and ascertained information about the organization of cervical collagen including the dominant orientation and distribution of cervical collagen. Such information may provide an indication of risk of preterm birth in pregnant women.

10043-3, Session 1

Novel multiplexed low coherence interferometry endoscopic probe for analyzing the cervical epithelium in vivo

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The cervix is primarily composed of two types of epithelium: stratified squamous ectocervix and simple columnar endocervix. In between these two layers lies a metaplastic squamocolumnar junction commonly referred to as the transformation zone (T-zone). During puberty, the cervical epithelium undergoes dynamic changes including cervical ectropion and increased area and rates of metaplasia. Although these metaplastic changes have been linked to higher incidence of cervical cancer among young women, research in this field has been limited to surface analysis using computerized planimetry of colopophotographs.

Here, we present a novel multiplexed low coherence interferometry (mLCI) system for interrogating the cervical epithelium. The system is comprised of 6 parallel Mach-Zehnder interferometers in a time-multiplexed configuration that increases throughput by 6-fold to realize a combined 36-channel acquisition. A custom designed endoscopic handheld probe is used to collect sparsely sampled, depth-resolved scattering intensity profiles (A-scans) from a large field of view (25 x 25 mm) on the cervical epithelium in vivo. The instrument incorporates white light imaging through a plastic fiber bundle to co-register the mLCI A-scans to colpophotographs which are analyzed by a clinician to manually segment the cervical epithelium. Our preliminary data shows significant differences in characteristic A-scans from endocervical and ectocervical epithelium. These results demonstrate the feasibility of using mLCI as both a research tool for studying the relationship between cervical ectopy and cancer as well as a clinical instrument for identifying the at-risk T-zone on the cervix in vivo as a means to improve biopsy targeting. Further analysis will be performed to develop an algorithm for distinguishing the mLCI A-scans of endocervical, ectocervical, and metaplastic epithelium in real time.

10043-4, Session 1

Assessing the biomechanical properties of the porcine vagina for customized vaginal stents

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In patients born without vaginas, stents are used to prevent scarring and shortening of newly created vaginas. However, most stents are adult-sized, and thus, cannot be used comfortably or safely in young patients. A correctly sized stent would reduce postoperative scat tissue formation, but the correct amount of stretch is not known. Here, we utilize optical coherence elastography (OCE) to assess the changes in the stiffness of adult and piglet (non-estrogenized) vaginal tracts (VT) as a function of stretch. Custom dilators were 3D printed and the porcine VTs were stretched 0, 50, 100, and 150% of their measured size for 24 hours (n=5 for each stretch). OCE measurements were made on the VTs along the axial and radial directions. Mechanical testing by strip extensiometry was also tested along these two directions for the same samples. There was a borderline insignificant difference in elastic wave group velocity between the piglet and adult VTs (P=0.07), where the piglet tissue was stiffer than the adult tissue. There was a general reversal of the stiffness anisotropy in the piglet tissue at 150%, which was not present in the adult tissue. Therefore, there may be an age, or possibly estrogen, dependence on the changes in biomechanical properties of the VT tissue as a function of stretch.

10043-5, Session 2

Image-guided therapeutics in experimental ovarian cancer *(Invited Paper)*

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The complexity of molecular processes in cancer development requires intelligent combination therapeutics that not only disable/enable the appropriate pathways, but do so at the right time. In this context photodynamic therapy (PDT) and optical imaging may have a special role. This paper will present out efforts at image-guided therapeutics and nanotechnology to optimize treatment outcomes.

10043-6, Session 2

Intra-operative label-free multi-modal multi-photon imaging of breast cancer margins and microenvironment

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Label-free multi-photon imaging has been a powerful tool for studying tissue microstructures and biochemical distributions, particularly for investigating tumors and their microenvironments. However, it remains challenging for traditional bench-top multi-photon microscope systems to conduct ex vivo tumor tissue imaging in the operating room due to their bulky setups and laser sources. In this study, we designed, built, and clinically demonstrated a portable multi-modal nonlinear label-free microscope system that combined four modalities, including two- and three- photon fluorescence for studying the distributions of FAD and NADH, and second and third harmonic generation, respectively, for collagen fiber structures and the distribution of micro-vesicles found in tumors and the microenvironment. Optical realignments and switching between modalities were motorized for more rapid and efficient imaging and for a light-tight enclosure, reducing ambient light noise to only 5% within the brightly lit operating room. Using up to 20 mW of laser power after a 20x objective, this system can acquire multi-modal sets of images over 600 ?m ? 600 ?m at an acquisition rate of 60 seconds using galvo-mirror scanning. This portable microscope system was demonstrated in the operating room for imaging fresh, resected, unstained breast tissue specimens, and for assessing tumor margins and the tumor microenvironment. This real-time label-free nonlinear imaging system has the potential to uniquely characterize breast cancer margins and the microenvironment of tumors to intraoperatively identify structural, functional, and molecular changes that could indicate the aggressiveness of the tumor.

10043-7, Session 2

Optical metabolic imaging measures early drug response in an allograft murine breast cancer model

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Previous work has shown that cellular-level Optical Metabolic Imaging (OMI) of organoids derived from human breast cancer cell-line xenografts accurately and rapidly predicts in vivo response to therapy. To validate OMI as a predictive measure of treatment response in an immune-competent model, we used the polyomavirus middle-T (PyVmT) transgenic mouse breast cancer model. The PyVmT model includes intra-tumoral heterogeneity and a complex tumor microenvironment that can influence treatment responses. Three-dimensional organoids generated from primary PyVmT tumor tissue were treated with a chemotherapy (paclitaxel) and a PI3K inhibitor (XL147), each alone or in combination. Cellular subpopulations of response were measured using the OMI Index, a composite endpoint of metabolic response comprised of the optical redox ratio (ratio of the fluorescence intensities of metabolic co-enzymes NAD(P)H to FAD) as well as the fluorescence lifetimes of NAD(P)H and FAD. Combination treatment significantly decreased the OMI Index of PyVmT tumor organoids ($p<0.0001$) and in vivo tumors ($p<0.0001$) versus controls. Subpopulation analyses revealed a homogeneous response to combined therapy in both cultured organoids and in vivo tumors, while single agent treatment with XL147 alone or paclitaxel alone elicited heterogeneous responses in organoids. Tumor volume decreased with combination treatment through treatment day 30. These results indicate that OMI of organoids generated from PyVmT tumors can accurately reflect drug response in heterogeneous allografts with both innate and adaptive immunity. Thus, this method is promising for use in humans to predict long-term treatment responses accurately and rapidly, and could aid in clinical treatment planning.

10043-8, Session 3

Cost-effective triaging of prostatectomy specimens using light sheet microscopy

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Pathology laboratories face competing pressures of the costs from tissue processing versus providing high quality care by thorough sampling of tissue specimens. Laboratories are reimbursed per-case rather than by work volume. Consequently, processing more tissue per case has a negative economic impact on the laboratory. Conversely, inadequate tissue sampling can adversely affect the patient?s care, i.e. false negative surgical margins, inaccurate tumor grading, and inaccurate tumor staging. Prostatectomy specimens represent an especially challenging tissue to sample since most prostate carcinomas are not grossly visible. Thus, a thorough sampling method that also limits laboratory workload is of great importance. We describe an inverted light sheet microscope (LSM) system for non-

destructive imaging of surgical resection specimens, using prostatectomy tissue as an example. The LSM system images a shallow depth (~50 microns) of resected tissue (up to 10x10 cm) at high speed (<1 min/cm^2) with cellular resolution (~2 microns). LSM-generated images are compared to corresponding H&E-stained slides for sensitivity and specificity analyses. We show that the LSM system allows for rapid, non-destructive classification of tissue as either positive or negative for carcinoma. Tissue classified as negative for carcinoma need not be submitted for processing, while carcinomatous tissue can be processed for detailed examination of H&E-stained slides. The LSM system presents the rare opportunity to decrease costs while maintaining high quality care, a feature of increasing importance in the evolving healthcare landscape.

10043-9, Session 3

Assessment of fresh, large breast tissue specimens with confocal strip-mosaicking microscopy

Sanjeewa Abeytunge, Memorial Sloan-Kettering Cancer Ctr. (United States); Bjorg A. Larson, Drew Univ. (United States); Gary Peterson, Milind Rajadhyaksha, Melissa Murray, Memorial Sloan-Kettering Cancer Ctr. (United States)

Confocal microscopy is in clinical use to diagnose skin cancers in the United States and in Europe. Potentially, this technology may provide bed-side pathology in breast cancer surgery during tumor removal. Initial studies have described major findings of invasive breast cancers as seen on fluorescence confocal microscopy. In many of these studies the region of interest (ROI) used in the analysis was user-selected and small (typically 15 square-mm). Although these important findings open exploration into rapid pathology, further development and implementation in a surgical setting will require examination of large specimens in a blinded fashion that will address the needs of typical surgical settings. In post surgery pathology viewing, pathologists inspect the entire pathology section with a low (2X) magnification objective lens initially and then zoomed in to ROIs with higher magnification lenses (10X to 40X) magnifications to further investigate suspected regions. In this study we explore the possibility of implementation in a typical surgical setting with a new microscope, termed confocal strip-mosaicking microscope (CSM microscope), which images an area of 400 square-mm (2 cm x 2 cm) of tissue with cellular level resolution in 10 minutes. CSM images of 34 human breast tissue specimens from 18 patients were blindly analyzed by a board-certified pathologist and correlated with the corresponding standard fixed histopathology. Invasive tumors and benign tissue were clearly identified in CSM images. Thirty specimens were concordant for images-to-histopathology correlation while four were discordant. Preliminary results from on-going work to molecularly target tumor margin will also be presented.

10043-10, Session 3

Breast cancer margin delineation with fluorescence lifetime imaging

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The current standard of care for early stages of breast cancer is breast-conserving surgery (BCS). BCS involves a lumpectomy procedure, during which the tumor is removed with a rim of normal tissue—if cancer cells found in that rim of tissue, it is called a positive margin and means part of the tumor remains in the breast. Currently there is no method to determine if cancer cells exist at the margins of lumpectomy specimens aside from time-intensive histology methods that result in reoperations in up to 38% of cases. We used fluorescence lifetime imaging (FLIm) to measure time-resolved autofluorescence from N=13 ex vivo human breast cancer specimens (N=10 patients undergoing lumpectomy or mastectomy) and compared our results to histology. Tumor (both invasive and ductal carcinoma in situ), fibrous tissue, fat and fat necrosis have unique fluorescence signatures. For instance, between 500-580 nm, fluorescence lifetime of tumor was shortest (4.7 ± 0.4 ns) compared to fibrous tissue (5.5 ± 0.7 ns) and fat (7.0 ± 0.1 ns), $P<0.05$ (ANOVA). These differences are due to the biochemical properties of lipid, nicotineamide adenine dinucleotide (NADH) and collagen fibers in the fat, tumor and fibrous tissue, respectively. Additionally, the FLIm data is augmented to video of the breast tissue with image processing algorithms that track a blue (450 nm) aiming beam used in parallel with the 355 nm excitation beam. This allows for accurate histologic co-registration and in the future will allow for three-dimensional lumpectomy surfaces to be imaged for cancer margin delineation.

10043-11, Session 3

Design of a portable, wide field of view, GPU-accelerated multiphoton imaging system for real-time surgical pathology

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Multiphoton microscopy is a promising method for performing intrasurgical evaluation of surgical specimen margins because of its molecular specificity and ability to optically section thick tissue. Previously, our group demonstrated high sensitivity and specificity for evaluating breast pathology post-operatively using multiphoton microscopy [1]. To facilitate clinical translation, we now describe the design and operation of a portable, inverted and wide field of view multiphoton system including microscope and femtosecond laser, operating on a 2.5 by 3 foot vibration-isolated cart suitable for operation in a clinic.

Combined with the new system, a new imaging protocol and a novel image processing algorithm are presented that render real-time virtual H&E histology images of fresh, unfixed tissue with dramatically improved imaging speed, contrast and correspondence with conventional H&E pathology as compared to previous methods. The combination of optimized scan optics and GPU-accelerated processing enable video rate, low latency virtual H&E histology of thick, unfixed tissue specimens at high resolution over a wide field of view. New staining protocols are discussed that enable more rapid specimen preparation and the use of alternative, compact light sources is presented. The system images a 2 mm x 2 mm field of view with 2048 x 2048 pixels at <1 um transverse resolution and a 8 Hz frame rate. In addition, fast motorized actuation enables visualization of many-centimeter scale samples at surgically relevant time scales. We discuss our experience operating the system in the clinic and optimizing workflows.

[1] Y. K. Tao, et al., "Assessment of breast pathologies using nonlinear microscopy," Proc. Natl. Acad. Sci., 2014.

10043-40, Session 3

Resection margin assessment during breast conserving surgery: Ex vivo spectroscopic characterization of invasive carcinoma and DCIS

Lisanne L. de Boer, Esther Kho, Koen Van de Vijver, Frederieke van Duijnhoven, The Netherlands Cancer Institute (Netherlands); Benno H. W. Hendriks, Koninklijke Philips N.V. (Netherlands) and Technische Univ. Delft (Netherlands); Henricus J.C. M. Sterenborg, Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek Ziekenhuis (Netherlands) and Academisch Medisch Centrum (Netherlands); Theo J. M. Ruers, Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek Ziekenhuis (Netherlands) and MIRA, Univ. Twente (Netherlands)

Re-excision is required in approximately 10-40% of patients that underwent breast conserving surgery due to incomplete resection. This includes both invasive carcinoma and ductal carcinoma in situ (DCIS). DCIS is a precursor stage of invasive carcinoma in which small volumes of tumor cells are still limited to the ducts. We investigated the optical differences between these cancerous tissue types and surrounding normal breast tissue using fiber-optic Diffuse Reflectance Spectroscopy (DRS).

DRS measurements (400-1600nm) were obtained from areas suspected for tumor or DCIS as well as normal tissue in 25 fresh breast surgery specimens. A custom-made frame was used to fixate the probe and allow correlation between measurement locations and histopathology afterwards. Analysis of the spectra was performed using an analytical fit model. This analytical model, based on the diffusion theory, quantified the measured spectra in optical parameters such as fat and water content.

Results show that differences between normal and tumor tissue are present in the near-infrared wavelength region (1000-1200nm) where fat and water have distinct absorption characteristics. Ten specimens had an invasive tumor according to the pathology report. In those specimens, measurement sites with invasive carcinoma could be discriminated from normal tissue based on fat and water content. Discrimination between normal tissue and DCIS proved more challenging, especially for pockets of DCIS that were small compared to the sampling volume of DRS. This issue related to the resolution of the measurements may be resolved by comparing the spectra obtained with different fiber distances.

10043-12, Session 4

Spatial frequency domain imaging (SFDI) as a new tool for monitoring chemotherapy response and resistance *(Invited Paper)*

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10043-13, Session 4

Improving breast cancer diagnosis by reducing chest wall effect in US-guided diffuse optical tomography

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We have developed ultrasound (US)-guided diffuse optical tomography (DOT) technique to assist US diagnosis of breast cancer and to predict neoadjuvant chemotherapy response of breast cancer patients. The technique was implemented using a handheld hybrid probe consisting co-registered US transducer and optical source and detector fibers which couple the light illumination from laser diodes and photon detection to PMT detectors. With the US guidance, diffused light measurements were made at the breast lesion site and the normal contralateral reference site which was used to estimate the background tissue optical properties for imaging reconstruction. However, background optical properties were affected by the chest wall underneath the breast tissue. In this study, we have analyzed data from 297 female patients and results have shown statistical significant correlation between fitted optical properties (μa and μs') and the chest wall depth detected by a boundary detection algorithm applied to co-registered US images ($r > 0.27$, $p < 1.0$? 10-4). After subtracting the background μa at each wavelength, the difference of computed total hemoglobin (tHb) between malignant and benign lesion groups has improved. The Area-under-the- ROC curve (AUC) has improved from 86.5% to 89.5% (sensitivity improved from 85.0% to 87.5% and specificity from 87.5% to 89.7%).

10043-14, Session 4

Automated adipose map generation for assessing cancerous human breast tissue using optical coherence tomography

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Breast cancer is the third leading cause of death in women in the United States. In human breast tissue, adipose cells are infiltrated or replaced by cancer cells during the development of breast tumor. Therefore, an adipose map can be an indicator of identifying cancerous region. We developed an automated classification method to generate adipose map within human breast.

To facilitate the automated classification, we first mask the B-scans from OCT volumes by comparing the signal noise ratio with a threshold. Then, the image was divided into multiple blocks with a size of 30 pixels by 30 pixels. In each block, we extracted texture features such as local standard deviation, entropy, homogeneity, and coarseness. The features of each block were input to a probabilistic model, relevance vector machine (RVM), which was trained prior to the experiment, to classify tissue types. For each block within the B-scan, RVM identified the region with adipose tissue. We calculated the adipose ratio as the number of blocks identified as adipose over the total number of blocks within the B-scan.

We obtained OCT images from patients (n = 19) in Columbia medical center. We automatically generated the adipose maps from 24 B-scans including normal samples (n = 16) and cancerous samples (n = 8). We found the adipose regions show an isolated pattern that in cancerous tissue while a clustered pattern in normal tissue. Moreover, the adipose ratio (52.30 ± 29.42%) in normal tissue was higher than the that in cancerous tissue (12.41 ± 10.07%).

10043-15, Session 4

Diffusion-Sensitive OCT using diffusive GNRs to monitor ECM remodeling by fibroblasts

Richard L. Blackmon, Rupninder Sandhu, The Univ. of North Carolina at Chapel Hill (United States); Brian S. Chapman, North Carolina State Univ. (United States);

Patricia Casbas, Ponce Health Sciences Univ. (United States); Joseph B. Tracy, North Carolina State Univ. (United States); Melissa A. Troester, Amy L. Oldenburg, The Univ. of North Carolina at Chapel Hill (United States)

Previous investigators have shown that remodeling of the extracellular matrix (ECM) changes the ECM pore size (porosity) which may contribute to the aggressiveness of breast cancer. Additionally, fibroblasts have been shown to remodel the ECM through mechanical force, and matrix protein secretion and degradation. Current methods of studying ECM properties require active mechanical deformation, and may not discriminate between ECM and cell stiffness. Here, we employ diffusion-sensitive OCT (DS-OCT) to passively measure the porosity of both collagen (2, 5, and 8mg/mL concentrations), and artificial ECM during remodeling by reduction mammoplasty fibroblasts (RMFs; 1, 100, 300, and 500cells/μL initial seed densities; incubated for 48 hours) in 2D cross-sections, with resolutions of 10?4.65μm (x?z). Due to their comparable size to ECM pores, the Brownian motion of PEG-coated gold nanorods (GNRs, 84?24nm) through ECM, quantified by their diffusion coefficient (Dt), is hindered as ECM porosity decreases. Measurements were validated against scanning electron microscopy (SEM), with measured diameters ranging from 275-615nm. From this data, we use a linear regression model to relate SEM-measured porosity and mean Dt in both collagen-only and artificial ECM matrices (R^2=0.97). We then employ DS-OCT to spatially resolve porosity of layered collagen matrices, and artificial ECM using RMF cell cultures, seeded with 0, 50, and 100cells/μL. We show that DS-OCT successfully discriminates the layers in collagen-only matrices. In RMF cell cultures, we show the pattern of ECM remodeling by DS-OCT for the first time, finding that the overall porosity decreases and spatial heterogeneity increases with increasing cell seed densities.

10043-16, Session 4

Rapid Mueller matrix polarimetry imaging for breast cancer analysis

Adam Gribble, Alex Vitkin, Univ. of Toronto (Canada)

Polarized light has many applications in biomedical imaging. The interaction of a biological sample with polarized light reveals information about its biological composition, both structural and functional. The most comprehensive type of polarimetry analysis is to measure the Mueller matrix, a polarization transfer function that completely describes how a sample interacts with polarized light. However, determination of the Mueller matrix requires tissue analysis under many different states of polarized light; a time consuming and measurement intensive process. Here we address this limitation with a new rapid polarimetry system, and use this polarimetry platform to investigate a variety of tissue changes associated with breast cancer.

We have recently developed a rapid polarimetry imaging platform based on four photoelastic modulators (PEMs). The PEMs generate fast polarization modulations that allow the complete sample Mueller matrix to be imaged over a large field of view, with no moving parts. This polarimetry system is then demonstrated to be sensitive to a variety of tissue changes that are relevant to breast cancer. Specifically, we show that changes in depolarization can reveal tumor margins, and can differentiate between viable and necrotic breast cancer metastasized to the lymph nodes. Furthermore, the polarimetric property of linear retardance (related to birefringence) is dependent on collagen organization in the extracellular matrix. These findings indicate that our polarimetry platform may have future applications in fields such as breast cancer diagnosis, improving the speed and efficacy of intraoperative pathology, and providing prognostic information that may be beneficial for guiding treatment.

10043-17, Session 5

Light-sheet microscopy for quantitative ovarian folliculometry

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The incidence of ovarian ailments such as premature ovarian failure (POF) and polycystic ovary syndrome (PCOS) has risen in recent years, constituting a major cause of female subfertility in the modern world, and common methods for diagnosis are follicle monitoring and determination of ovarian status. Conventional folliculometry with histology is limited due to the irregularity in follicle geometry.

We evaluated the suitability of selective plane illumination microscopy (SPIM) for the study of porcine ovarian follicles. The large field of view and fast acquisition speed of the SPIM system enables rendering of volumetric image stacks from intact whole porcine ovarian follicles, clearly visualizing follicular features including follicle diameter (70 ?m - 2.5 mm), size of developing cumulus oophorus complexes (40 ?m - 110 ?m), and follicular wall thickness (90 ?m-120 ?m). Volumetric analysis of follicle size allowed identification of different folliculogenesis stages, even small primordial follicle clusters forming egg nests. Other morphological parameters were quantified, including follicle asymmetry and the distribution of theca thickness. For both parameters, inverse correlations with follicle developmental stage were found.

The ability of the SPIM system to non-destructively generate sub-cellular resolution 3D images of developing follicles, with excellent image contrast and high throughput capacity compared to conventional histology, suggests that it can be used to monitor follicular development and identify structural abnormalities indicative of ovarian ailments. Accurate folliculometric measurements provided by SPIM images can immensely help the understanding of ovarian physiology and provide important information for the proper management of ovarian diseases.

10043-18, Session 5

A dual-modality optical coherence tomography and selective plane illumination microscopy system for mouse embryonic imaging

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The mouse embryo is the most common animal model for studying human congenital disease. A multi-modality imaging technique that integrates complementary optical information can provide more comprehensive tissue characterization for mouse embryonic research. Optical coherence tomography (OCT) is a high resolution cross-sectional imaging technique and rotational imaging optical coherence tomography (ri-OCT) is a technical improvement of OCT to increase the imaging field. Selective plane illumination microscopy (SPIM) is a fluorescence microscopy technique that uses a focused light sheet to illuminate a plane of the specimen. In this study, we have combined ri-OCT and SPIM into a single dual-modality

device to image E9.5 stage mouse embryos. A digital image manipulation method was developed to fuse the images acquired from different modalities. This provides the unique advantage of simultaneously collecting a comprehensive time-resolved 3D image of the embryo with antigen-specific labels. The results demonstrate that the dual-modality setup is able to characterize the mouse embryo morphology and vasculature in developing embryos simultaneously.

10043-19, Session 5

Bessel beam Fourier multiplexed FLIM tomography for 3D embryonic imaging at cellular resolution

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Fourier multiplexed fluorescence lifetime imaging (FmFLIM) scanning laser optical tomography (FmFLIM-SLOT) combines FmFLIM and Scanning laser optical tomography (SLOT) to perform multiplexed 3D FLIM imaging of live embryos. The system had demonstrate multiplexed functional imaging of zebrafish embryos genetically express Foster Resonant Energy Transfer (FRET) sensors.

However, previous system has a 20 micron resolution because the focused Gaussian beam diverges quickly from the focused plane, makes it difficult to achieve high resolution imaging over a long projection depth. Here, we present a high-resolution FmFLIM-SLOT system with achromatic Bessel beam, which achieves 3 micron resolution in 3D deep tissue imaging.

In Bessel-FmFLIM-SLOT, multiple laser excitation lines are firstly intensity modulated by a Michelson interferometer with a spinning polygon mirror optical delay line, which enables Fourier multiplexed multi-channel lifetime measurements. Then, a spatial light modulator and a prism are used to transform the modulated Gaussian laser beam to an achromatic Bessel beam. The achromatic Bessel beam scans across the whole specimen with equal angular intervals as sample rotated. After tomography reconstruction and the frequency domain lifetime analysis method, both the 3D intensity and lifetime image of multiple excitation-emission can be obtained.

Using Bessel-FmFLIM-SLOT system, we performed cellular-resolution FLIM tomography imaging of live zebrafish embryo. Genetically expressed FRET sensors in these embryo will allow non-invasive observation of multiple biochemical processes in vivo.

10043-20, Session 5

Prevention of congenital defects induced by prenatal alcohol exposure

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Over 500,000 women per year in the United States drink during pregnancy, and 1 in 5 of this population also binge drink. Up to 40% of live-born children with prenatal alcohol exposure (PAE) present with congenital heart defects (CHDs) including life-threatening outflow and valvuloseptal anomalies. Previously we established a PAE model in the avian embryo and used optical coherence tomography (OCT) imaging to assay looping-stage (early) cardiac function/structure and septation-stage (late) cardiac defects. Early-stage ethanol-exposed embryos had smaller cardiac cushions (valve precursors) and increased retrograde flow, while late-stage embryos presented with gross head/body defects, and exhibited smaller atrio-ventricular (AV) valves, interventricular septae, and aortic vessels. However, supplementation with the methyl donor betaine reduced gross defects, prevented cardiac defects such as ventricular septal defects and abnormal AV valves, and normalized cardiac parameters. Immunofluorescent staining for 5-methylcytosine in transverse embryo sections also revealed that DNA methylation levels were reduced by ethanol but normalized by co-administration of betaine. Furthermore, supplementation with folate, another methyl donor, in the PAE model appeared to normalize retrograde flow levels which are typically elevated by ethanol exposure. Studies are underway to correlate retrograde flow numbers for folate with associated cushion volumes. Finally, preliminary findings have revealed that glutathione, a key endogenous antioxidant which also regulates methyl group donation, is particularly effective in improving alcohol-impacted survival and gross defect rates. Current investigations will determine whether glutathione has any positive effect on PAE-related CHDs. Our studies could have significant implications for public health, especially related to prenatal nutrition recommendations.

10043-21, Session 5

Quantification and visualization of injury and regeneration in the developing ciliated epithelium using quantitative flow imaging and speckle variance optical coherence tomography

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Premature infants are at a high risk for respiratory diseases owing to an underdeveloped respiratory system that is very susceptible to infection and inflammation. One aspect of respiratory health is the state of the ciliated respiratory epithelium which lines the trachea and bronchi. The ciliated epithelium is responsible for trapping and removing pathogens and pollutants from the lungs and an impairment of ciliary functionality can lead to recurring respiratory infections and subsequent lung damage. Mechanisms of cilia-driven fluid flow itself but also factors influenced by development like ciliary density and flow generation are incompletely understood. Furthermore, medical interventions like intubation and accidental aspiration can lead to focal or diffuse loss of cilia and disruption of flow. In this study we use two animal models, Xenopus embryo and ex vivo mouse trachea, to analyze flow defects in the injured ciliated epithelium. Injury is generated either mechanically with a scalpel or chemically by calcium chloride (CaCl2) shock, which efficiently but reversibly deciliates the embryo skin. In this study we used optical coherence tomography (OCT) and particle tracking velocimetry (PTV) to quantify cilia driven fluid flow over the surface of the Xenopus embryo. We additionally visualized damage to the ciliated epithelium by capturing 3D speckle variance images that highlight beating cilia. Mechanical injury disrupted cilia-driven fluid flow over the injured site, which led to a reduction in cilia-driven fluid flow over the whole surface of the embryo (n=7). The calcium chloride shock protocol proved to be highly effective in deciliating embryos (n=6). 3D speckle variance images visualized a loss of cilia and cilia-driven flow was halted immediately after application. We also applied CaCl2-shock to cultured ex vivo mouse trachea (n=8) and found, similarly to effects in Xenopus embryo, an extensive loss of cilia with resulting cessation of flow. We investigated the regeneration of the ciliated epithelium after an 8 day incubation period, and found that cilia had regrown and flow was completely restored. In conclusion, OCT is a valuable tool to visualize injury of the ciliated epithelium and to quantify reduction of generated flow. This method allows for systematic investigation of focal and diffuse injury of the ciliated epithelium and the assessment of mechanisms to compensate for loss of flow.

10043-35, Session 5

In vivo imaging and analysis of reproductive activities in the mouse oviduct using optical coherence tomography

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Visualization of the dynamic reproductive process inside the mouse oviduct is essential to advance the understanding of mammalian reproduction and improve the management of reproductive disorders, such as infertility and ectopic pregnancy. In vitro or ex vivo investigations of the mammalian reproductive tract give limited understanding about dynamic events that naturally occur during reproductive system development, estrous cycle and pre-implantation pregnancy. As a result, fundamental reproductive processes, such as ovulation, fertilization and pre-implantation development, are poorly understood from dynamic point of view. To address this lack of knowledge, we employed optical coherence tomography (OCT) and developed a set of OCT-based imaging methods for in vivo structural, dynamic and functional visualization of features of the mouse oviduct, which previously have not been accessible. The micro-scale spatial resolution, millimeter-level imaging depth, large transverse field of view, high temporal resolving ability, functional capacity and compatibility with live imaging make this approach applicable for a variety of reproductive studies. We are now taking these methods into analysis of abnormal mutant phenotypes to understand genetic effects on dynamic reproductive processes.

10043-22, Session 6

Gender differences in heart morphology and function of wild type Drosophila melanogaster at different developmental stages

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The Drosophila melanogaster shares many similarities with vertebrates in heart development. Comparison of heart structural and functional characteristic between male and female Drosophila melanogaster at different developmental stages is helpful to understand heart morphogenesis and function for different genders. And also, it opens up the possibility to uncover the role of sex-related genes in heart development. In this longitudinal study, we cultured and tracked dozens of individually labeled flies throughout their lifecycle. The heart characteristic was measured at different developmental stages during culturing. The gender of each individual fly was determined by adult stage so that the collected data of early stages could be classified to male or female group. We adapted a high-speed optical coherence microscopy (OCM) system with axial and transverse resolution of 2um and 4um, respectively, to perform non-invasive M-mode imaging at a frame rate of 132Hz in Drosophila heart at third instar larva, early pupa and adult stage. Based on those GPU processed M-mode OCM images, we segmented the fly heart region and then quantified the cardiac structural and functional parameters such as heart rate, heart chamber size and so on. Despite large variances of wild type Drosophila in terms of some cardiac characteristic, our results suggest that the heart rate is lower for male flies than for female flies, especially at third instar larva stage. The end diastolic area (EDA) and end systolic area (ESA) of the heart are both slightly larger in female flies than in male flies at larva and adult stage. In summary, we showed gender differences of wild type drosophila in heart functional and structural characteristic.

10043-23, Session 6

live dynamic analysis of the developing cardiovascular system in mice

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The study of the developing cardiovascular system in mice is important for understanding human cardiogenesis and congenital heart defects. Our research focuses on imaging early development in the mouse embryo to specifically understand cardiovascular development under the regulation of dynamic factors like contractile force and blood flow using optical coherence tomography (OCT). We have previously developed an OCT based approach that combines static embryo culture and advanced image processing with computational modeling to live-image mouse embryos and obtain 4D (3D+time) cardiodynamic datasets. Here we present live 4D dynamic blood flow imaging of the early embryonic mouse heart in correlation with heart wall movement. From our data, we observed distinctive heart wall dynamics suggesting a unique role for retrograde flow. We are using this approach to understand how specific mutations impact heart wall dynamics, and how this influences flow patterns and cardiogenesis. We perform studies in mutant embryos with cardiac phenotypes such as intraflagellar transport (Ift) mutants and myosin regulatory light chain 2, atrial isoform (Mlc2a). This work is bringing us closer to understanding the connections between dynamic mechanical factors and gene programs responsible for early cardiovascular development.

10043-24, Session 6

Ex vivo cell counting in whole mount tissue volumes using expansion optical coherence tomography (OCT)

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Abnormal cell proliferation and migration during heart development can lead to severe congenital heart defects (CHDs). Studying the spatial distribution of cells during embryonic development helps our understanding of how the heart develops and the etiology of certain CHDs. However, imaging large groups of single cells in intact tissue volumes is challenging. No current technique can accomplish this task in both a time-efficient and cost-effective manner. OCT has potential with its large field of view and micron-scale resolution, but even the highest resolution OCT systems have poor contrast for counting cells and have a small field of view compared to conventional OCT. We propose using a conventional OCT system and processing the sample to enhance cellular contrast. Inspired by the recently developed Expansion Microscopy, we permeated whole-mount embryonic tissue with a superabsorbent monomer solution and polymerized into a hydrogel. When hydrated in DI water, the tissue-hydrogel complex was uniformly enlarged (~5X in all dimensions) without distorting the microscopic structure. This had a twofold effect: it increased the resolution by a factor of 5 and decreased scattering, which allowed us to resolve cellular level features deep in the tissue with high contrast using conventional OCT. We noted that cell nuclei caused significantly more backscattering than the other subcellular structures after expansion. Based on this property, we were able to distinguish individual cell nuclei, and thus count cells, in expanded OCT images with simple intensity thresholding. We demonstrate the technique with embryonic quail hearts at various developmental stages.

10043-25, Session 6

Automated assessment of blood flow in developing embryonic hearts by extending dynamic range of Doppler OCT using a MHz FDML swept laser source

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Altered hemodynamics in developing embryonic hearts lead to congenital heart diseases, motivating close monitoring of blood flow over several stages of development. Doppler OCT can assess blood flow in tubular hearts, but the maximum velocity increases drastically during the period of cardiac cushion (valve precursors) formation. Therefore, the limited dynamic range of Doppler OCT velocity measurement makes it difficult to conduct longitudinal studies without phase wrapping at high velocities or loss of sensitivity to slow velocities. We have built a high-speed OCT system using an FDML laser (Optores GmbH, Germany) at a sweep rate of 1.68 MHz (axial resolution - 12 ?m, sensitivity - 105 dB, phase stability - 17 mrad). The speed of this OCT system allows us to acquire high-density B-scans to obtain an extended velocity dynamic range without sacrificing the frame rate. The extended dynamic range within a frame is achieved by varying the A-scan interval at which the phase difference is found, enabling detection of velocities ranging from tens of microns per second to hundreds of mm per second. The extra lines in a frame can also be utilized to improve the structural and Doppler images via complex averaging. In structural images where presence of blood causes additional scattering, complex averaging helps retrieve features located deeper in the tissue. Moreover, high-density frames can be registered to 4D volumes to determine the orthogonal direction of flow and calculate shear stress. In conclusion, our high-speed OCT system will enable automated Doppler imaging of embryonic hearts in cohort studies.

10043-26, Session 6

Studying the development of early post-implantation mouse embryos by using microCT and lightsheet microscopy

Chih-Wei Hsu, Henry H. Le, Nanbing Li-Villarreal, Victor G. Piazza, Sowmya Kalaga, Mary E. Dickinson, Baylor College of Medicine (United States)

Hemodynamic force is vital to cardiovascular remodeling in the early post-implantation mouse embryo. Here, we present work using microCT and lightsheet microscopy to establish the critical sequence of developmental events required for forming functional vasculature and circulation in the embryo, yolk sac, and placenta in the context of normal and impaired flow. A flow impaired model, Mlc2a+/- will be used to determine how hemodynamic force affects the specific events during embryonic development and vascular remodeling between the 4 and 29-somite stage using microCT. We have recently established high-resolution methods for the generation of 3D image volumes from the whole embryo within the deciduum (Hsu et al., in revision). This method enables the careful characterization of 3D images of vitelline and umbilical vessel remodeling to define how poor blood flow impacts both vitelline and umbilical vessel remodeling. Novel lightsheet live imaging techniques will be used to determine the consequence of impaired blood flow on yolk sac vasculature remodeling and formation of umbilical vessels using transgenic reporters: Flk-myr::mCherry, Flk1-H2B::YFP, or ?Globin-GFP. High-resolution 3D imaging of fixed and ScaleA2-cleared whole mount embryos labeled with Ki67 and Caspase3 will also be performed using lightsheet microscopy to quantify the proliferation and apoptotic indexes of early post-implanted embryos and yolk sac. This multi-modality approach is aimed at revealing further information about the cellular mechanisms required for proper vessel remodeling and the initial stages in placentation during early post-implantation development.

10043-27, Session PSun

Application of Mueller matrix polarimetry and second harmonic imaging in the determination of uterine-cervix collagen orientation and distribution

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Preterm birth (PTB) presents a serious medical heath concern throughout the world. There is a high incidence of PTB in both developed and developing countries ranging from 11%-15%, respectively. Studies have shown there may be numerous precursors to PTB including infections, genetic predisposition, nutrition and various other morbidities which all lead to a premature disorganization in the cervical collagen resulting in the weakening of the structure designed to keep the fetus in utero. The changes in cervical collagen orientation and distribution may prove to be a predictor of PTB. Polarization imaging is an effective means to measure optical anisotropy in birefringent materials such as those rich in collagen as the cervix is. Non-invasive, full-field Mueller Matrix polarimetry (MMP) imaging methodologies and ex-vivo second harmonic generation (SHG) imaging were used to assess cervical collagen content and structure in non-pregnant porcine cervices. The SHG microscopy was used to verify the efficacy of the MMP in assessing changes in collagen orientation.

10043-28, Session PSun

Development of time-resolved diffuse optical tomography for breast cancer image

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In recent year, cancer is one of the most deadly diseases worldwide. For women, breast cancer is the second most common cancer after skin cancer. However, early diagnosis of breast cancer are still difficult for now. So we want to develop an optical system which is the near-infrared time-resolved diffuse optical tomography (NIR-TRDOT) system to assist doctors to diagnose breast cancer. The time-resolved diffuse optical tomography (TRDOT) contains two picosecond diode lasers (dual-wavelength near-infrared source) and two fast-gated single-photon avalanche diode (SPAD) (coupled to a time-correlated single-photon counting detectors). The advantages of TRDOT are non-invasive, non-radiation and fast imaging. When patients use this system, it would make them much comfortable relative to using other systems, such as X-rays, PET, MRI and so on. There are two channels which have four sources and two detectors. Simplify the process of calculation by Mellin-Laplace transform (MLT). We calculate absorption coefficients by solving the variance of the measured distributions of times of flight of photons (DTOFs). And then, we can put these coefficients into the time-domain diffusion equation to build the breast cancer image. This system could get the different depth information in tissue. The absorption coefficient of breast cancer is higher than normal breast tissue so that we could know when the patient have breast cancer, the absorption coefficient of breast cancer will be different than normal ones. NIR-TRDOT system could be used to diagnose breast cancer much more accurately.

10043-29, Session PSun

Side-by-side comparison between confocal and multiphoton microscopy for the evaluation of unfixed human surgical specimens

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Histopathology is the gold standard for definitive diagnosis of most types of cancer. However, the process of histopathology is time and labor consuming because it requires fixation and physical sectioning. Optical sectioning is a promising technique for rapid histopathological evaluation because it enables examination of cellular details of unfixed human tissue without physical sectioning. Few studies have compared available optical sectioning microscopies such as multiphoton microscopy (MPM) and confocal fluorescence microscopy (CFM). In this study, same unfixed human tissue specimens are examined under both MPM and CFM, and compared with conventional hematoxylin and eosin histology. We show that MPM is a promising candidate for the rapid histopathological evaluation because of low background signal from out of focus light, high axial resolution, and depth imaging capability. In addition, confocal microscopy is another promising candidate for the rapid evaluation of unfixed human tissue because of axial resolution comparable with conventional histology and low cost light source. The results demonstrate the imaging capability of histological details of unfixed human tissue by using commonly available representatives of MPM and CFM. Examination of various types of human tissue is conducted, and multiple staining protocols are also tested. The results suggest that both MPM and CFM will be able to generate images which are compatible with conventional histology, but optimization of imaging protocol (especially staining protocol) will be needed for each application.

10043-30, Session PSun

Quantifying the spatial distribution of metabolic heterogeneity in breast cancer

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Tumor heterogeneity has been identified as a primary factor driving treatment resistance in patients. Fluorescence lifetime imaging (FLIM) of autofluorescent metabolic co-enzymes NAD(P)H and FAD can image cell-level metabolic heterogeneity within tumors. Specifically, the optical metabolic imaging (OMI) index is a linear combination of the mean NAD(P)H lifetime, mean FAD lifetime, and the redox ratio (fluorescence intensity of NAD(P)H divided by that of FAD). Previously, the OMI index has been used to resolve responsive and resistant cancer cells within mixed samples. Here, an algorithm has been developed to quantify the spatial distribution of these tumor cell sub-populations. The algorithm was validated with respect to manual classification in co-cultures of MDA-MB-231 and SKBr3 breast carcinoma lines and applied to three-dimensional organoids generated from HR6 xenografts, associated with increased heterogeneity. Gaussian mixture modeling based on OMI index defined subpopulations in all heterogeneous samples. Descriptive spatial metrics were developed using density estimation integrated with standard image analysis. Within organoids, cells belonging to the same metabolic sub-population were clustered together, as ≥75% of neighboring cells were from the same sub-population. Drug responsive cells formed denser clusters than drug resistant cells, with shorter intercellular distances within responsive populations ($p<0.05$) compared to distances within resistant populations or distances between populations. Furthermore, both resistant and responsive subpopulations were dispersed equidistance from the center of mass of the organoid (52.0± 20.1 ?m and 62.1±28.7 ?m, respectively). Overall, these efforts to quantify spatial distribution of tumor cell sub-populations could provide valuable insight into tumor growth dynamics and treatment resistance.

10043-31, Session PSun

Optical coherence microscopy with extended focus for in vivo embryonic imaging

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Optical coherence microscopy (OCM) has unique advantages of high-resolution volumetric imaging without relying on exogenous labels or dyes. It combines the coherence-gated depth discrimination of optical coherence tomography (OCT) with the high lateral resolution of confocal microscopy, offering an excellent balance between resolution and imaging depth. However, as the lateral resolution becomes higher, the imaging depth of OCM decreases and its three-dimensional imaging capability is greatly degraded.

To overcome this limitation, we utilized amplitude apodization to create quasi-Bessel beam illumination in order to extend the depth of focus. Compared with methods of full Bessel beam, our approach improves the sensitivity of system and reduces the sidelobe artifacts. The lateral and axial resolutions of our OCM system were measured to be 1.4 μm and 2.8 μm in tissue, respectively. The imaging depth of field was characterized, showing ~3X extension (~50 μm) compared to the regular Gaussian beam illumination (~16 μm). Using zebrafish embryos as a test system, we demonstrate the extended-focus OCM for structural and functional imaging studies revealing the detailed anatomy of embryos and blood flow in their microvasculature. Thanks to the extended imaging depths, an iterative deconvolution algorithm can work better over larger depths to further improve the quality of OCM images.

10043-32, Session PSun

Brillouin microspectroscopy assessment of tissue differentiation during embryonic development

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Changes in mechanical properties represent one of the driving factors behind cell differentiation during embryonic development. However, measuring these changes without disrupting the normal progression of morphogenesis or destroying the developing organism is not trivial. Brillouin microspectroscopy has been shown to be capable of no-contact, non-destructive and non-disruptive assessment of elastic properties in developing zebrafish embryos. The present study builds upon the previous work, and observes the changes in elasticity during the development of heart and brain in zebrafish embryos from 8 to 28 hours post-fertilization at regular intervals. Brillouin microspectroscopy has proved to be a suitable technique to continuously monitor tissue differentiation and the development of individual organs with high spatial resolution without harming the developing organism.

10043-33, Session PSun

Peroperative imaging of human fallopian tubes using endoscopic optical coherence tomography and fluorescence spectroscopy for ovarian cancer detection

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In women with hereditary risks of ovarian cancer, prophylactic surgery is considered as a risk-reducing strategy. Women with suspicious diagnosis of tubo-ovarian lesions undergo debulking surgery. It has been shown that the majority of high grade serous carcinoma arise from fallopian tubes mucosa. Pathological examination should now include thorough examination of the excised tubes and ovaries. Even with total submission of fallopian tubes today, microscopic foci of serous tubal intraepithelial carcinoma could be missed during submission and technical preparation of material for pathological evaluation. We present an endoscopic Optical Coherence Tomography (OCT) system allowing for internal visualization of human fallopian tubes. This endoscopic imaging system has two custom interchangeable probes: the first with 1.2 mm outside diameter (static optics) and the second with 2.4mm outside diameter (rotating micro-motor). Both probes have axial resolutions of 12μm in air, lateral resolutions < 40μm and imaging depth around 2mm. We also used a custom-built fluorescence spectroscopy probe to collect autofluorescence signals locally (6 excitation sources between 390nm and 640nm).

A pilot study on 25 women undergoing either prophylactic or debulking surgeries was performed using both OCT imaging probes as well as fluorescence spectroscopy. Corresponding histologies were taken in 5 locations on each tubes. Imaging was performed between surgical removal and histological examination. The static probe was used to visualize the internal fallopian tube in its whole length whereas the rotating probe was used to image from the infundibulum to the fimbriated end. Fluorescence measurements were taken in the fimbriated end, infundibulum and ampulla.

10043-34, Session PSun

Ultrasound-guided spectral photoacoustic imaging of placental oxygenation

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Preeclampsia is a leading cause of fetal and maternal mortality. There are no therapies—beyond premature delivery of the placenta and fetus—to halt progression of the disease. Abnormal placental oxygenation, as a result of abnormal placentation, is thought to be an early step in the development of preeclampsia. Available clinical imaging methods are not capable of detecting changes in placental oxygenation, which could be used to predict patients at high risk of preeclampsia. We are developing ultrasound-guided spectral photoacoustic imaging methods to quantify placental oxygenation indicative of the progression of preeclampsia. In these studies, algorithms were developed to correlate the spectral photoacoustic data acquired using a Vevo LAZR small animal imaging system to a hemoglobin oxygenation (%sO2) calibration standard, consisting of a phantom containing blood at varying partial pressures of oxygen. Pregnant SWV mice were imaged longitudinally from E12.5 to 18.5. An overlay of the %sO2 on the ultrasound image maps placental variation in %sO2 during longitudinal development. We demonstrate a method to calibrate spectral photoacoustic data to %sO2 and apply that method to the analysis of placental %sO2 in vivo. We are applying these methods to the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia, to determine whether changes in placental oxygenation precede the development of detectable changes in blood pressure and proteinuria—indicating preeclampsia—in this preclinical model of preeclampsia. The developed imaging methods hold great potential for clinical translation for the early prediction of the development of preeclampsia.

10043-36, Session PSun

Structured Light Profilometry for Designated Breast Surface Coordinate Variation Analysis

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Early detection of breast carcinoma is vital for effective treatment option and to enhance the survival rate. Existing breast imaging methods such as mammography, ultrasound and MRI have been utilized for early detection of breast carcinoma which requires contact to the breast surface. However, these existing methods require contact to the breast surface which causes discomfort to the test subject.Hence, there is a need for alternative modality, which exhibits a total non contact nature. Structured light profilometry has developed into a vital system with its application in diverse fields of surface metrology analysis. Therefore, in this work structured light profilometry based on phase shift technique is setup to analyze the surface variation of breast due to presence of lesion in the context of surface tension. Sinosoidal fringe pattern are projected through three step phase shift on to the surface of the breast and a resulting phase map is produced. Pixel tracing was performed to evaluate the variation of surface changes on the breast based on surface marker coordinates. Comparison was done between breast with lesion and breast without lesion. Maiden results have established that the structured light profilometry is capable in detecting breast surface changes at various location on the breast.

10043-37, Session PSun

3D Mapping of Breast Surface Using Digital Fringe Projection

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Optical sensing technique has inherited non contact nature for generating 3D surface mapping where its application ranges from MEMS component characterization, corrosion analysis and vibration analysis. In particular, digital fringe projection are utilized for 3D mapping of objects through illumination of structured light for medical application ranging from oral dental measurements, lower back deformation analysis, monitoring of scoliosis and 3D face reconstruction for biometric identification. However, the usage of digital fringe projection for 3D mapping of human breast is very minimal. Thus, this paper addresses the application of digital fringe projection for 3D mapping of breast surface based on total non contact nature. In this work, phase shift technique is applied to perform the 3D

mapping. Phase shifted fringe pattern are projected through digital projector on to the breast surface and the deformed fringe patterns are recorded through a CCD camera. A phase map is produced and phase unwrapping was performed to obtain the 3D surface mapping of the breast. Calibration was performed on the projector and camera to further improve the output of 3D surface mapping of the breast. Preliminary results showed the feasibility of digital fringe projection in providing 3D mapping of breast and its application can be further extended for breast carcinoma detection.

10043-38, Session PSun

Detecting breast cancer using contrast-enhanced multimodal optical imaging

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Accurate demarcation of cancer margins is of major importance in surgical management of breast cancer. The goal of this study was to evaluate a molecular marker, pH low insertion peptide (pHLIP) for ex vivo intraoperative detection of breast cancer and compare the results with those yielded by an FDA-approved Methylene Blue (MB).

Breast tissue samples were obtained following surgery. They were immediately stained with pHLIP-Alexa532. Then the specimens were rinsed in aqueous MB. After staining, the samples were imaged with multimodal wide-field and confocal systems. Reflectance images were acquired between 390 nm and 750 nm. Fluorescence of pHLIP-Alexa-532 and MB was excited at 532 nm and 640 nm, respectively. Then the specimens were processed for H&E histopathology. Histological slides were digitized and compared with the wide-field and high-resolution optical images to evaluate correlation of tumor margins and cellular morphology, respectively.

Wide-field imaging demonstrated increased pHLIP-Alexa 532 fluorescence emission and high MB fluorescence polarization in cancerous regions. High-resolution imaging showed that both pHLIP-Alexa532 and MB accumulated in cancer cells. However, MB also stained nuclei of normal cells. As expected, fluorescence emission of MB highlights morphology of breast tissue and can be interpreted similarly to H&E histopathology. In contrast, pHLIP-Alexa532 stained the cytoplasm of cancer cells. Even though some fluorescence emission was detected from collagen and benign cell membranes, the results indicate that fluorescence emission imaging of pHLIP-Alexa 532 exhibits high specificity for breast cancer and can potentially be used for differentiation of malignant cancers from benign tumors and normal tissue.

10043-39, Session PSun

Rare-earth doped nanocomposites enable multiscale targeted short-wave infrared imaging of metastatic breast cancer

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Fluorescent rare earth (RE)-doped nanoparticles can be excited with near-infrared light (980 nm) to generate optical emissions in the short-wave infrared (SWIR) spectral region (1,000-1,700 nm). These spectral properties take advantage of reduced optical scattering and low autofluorescence to provide deeper imaging capability than visible or near infrared agents. Encapsulation of rare earth nanoparticles in human serum albumin renders these materials biocompatible and capable of displaying targeting ligands for molecular-specific imaging.

We are investigating the ability of targeted RE nanocomposites to detect and track micrometastatic breast cancer lesions to distant sites in pre-clinical in vivo mouse models. Functionalizing RE nanocomposites with AMD3100 promotes targeting to CXCR4, a recognized marker for highly metastatic disease. Mice were inoculated with SCP-28 (CXCR4 positive) and 4175 (CXCR4 negative) cell lines. Whole animal SWIR fluorescence imaging was performed over a four week time course, enabling early micrometastatic lesions to be identified and tracked to the lungs. In vivo MRI and bioluminescence imaging confirmed tumor burden. At endpoint, line-scanning confocal fluorescence microscopy provided high-resolution imaging of RE nanocomposite uptake in ex vivo tissue sections. Optical coherence tomography allowed assessment of tissue microarchitecture and micro lesions. Conventional H&E stained pathology sections were prepared for confirmation of metastatic spread.

RE-doped nanocomposites can be targeted to cancer biomarkers and deployed for multiscale tissue molecular imaging in pre-clinical models. Our ongoing studies are using these tools to further elucidate the mechanisms driving breast cancer metastasis and apply the findings to clinical patient care.

10043-41, Session PSun

Combined glucose and lipid metabolism reveals the correlation of cancer metabolism and invasiveness

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Warburg metabolism is characterized by aerobic glycolysis which is believed to be beneficial for glucose-based synthesis of biomolecules necessary for supporting cancer cell proliferation. Research has been done to use cellular redox state as a biomarker for cancer diagnosis and predict drug response. However, glucose metabolism, alone, is not sufficient enough to fully characterize cancer cells. Here, we demonstrate that by monitoring both glycolytic and lipid metabolic signatures, cancer cells can be differentiated based on their invasiveness. We used label-free nonlinear optical microscopy to quantitatively monitor live cell metabolism in 3D breast cancer models of primary mammary epithelial (PME) cells, T47D and MDA-MB- 231 cells. The three different cell lines can be differentiated based on their glucose and lipid metabolic signatures using multi-modal non-linear optical microscopy. Upon treating cells with 17?-estradiol (E2), both normal cells and ER-positive breast cancer cells exhibit an increased glycolysis rate. However, normal cells display increased lipid content while the lipid storage in cancer cells decreased ($p<0.01$) accompanied with metastatic transformation. Furthermore, we observe an increase in lipid synthesis and consumption rate in T47D cancer cells when treated with deuterium labeling. These results demonstrate an unambiguous correlation between cancer invasiveness and accessible cellular metabolic observables, as reported by glycolytic rate, lipid synthesis and lipid consumption.

10044-1, Session 1

Assessment of dental tissues and restorations using ultra-high resolution polarization-sensitive spectral-domain OCT

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Optical coherence tomography (OCT) has gained increasing interest as a potential diagnostic tool in dentistry due to its non-invasive and safe ability to obtain real-time high-resolution cross-sectional images. Typically, OCT is used at 1300nm wavelength due to its larger penetration depth compared to that at shorter wavelengths. However, we postulate that the ultra-high-resolution afforded by 800nm wavelength OCT (<3μm) may reveal new insights into restorative dentistry. Microcrack and void formation has been shown to be directly related to water sorption and hydrolytic degradation in resin-based dental materials (RBDMs). These defects negatively affect the mechanical properties and longevity of esthetic restorations. Also, marginal gaps at the tooth-restoration interface can potentially lead to recurrent caries. Here, we employ ultra-high resolution (1.9?10μm, x?z in dental tissue) polarization-sensitive, spectral-domain OCT (PS-SD-OCT) to detect microcracks and voids throughout RBDMs and evaluate marginal gaps between restorations and the tooth after mechanical stress. Restorations were present on the facial, lingual, mesial, and distal surfaces of each tooth. SD-OCT scans were collected over 3(or 4)?3(or 4)?1.34mm (x?y?z, n=120(or 160) images/scan). Our results show that microcracks and voids in RBMs can be influenced by both restorative techniques and dental material properties. Ultra-high-resolution PS-SD-OCT is effective in identifying the depth (z) and extent (x,y) of marginal gaps. Additionally, polarization sensitivity permitted easier differentiation of dental tissues and resin-based restorations, and allowed for reduction of surface reflection artifacts. In conclusion, ultra-high-resolution SD-OCT using a PS approach represents a promising diagnostic tool for restorative dentistry.

10044-2, Session 1

Performance comparison of optical coherent tomography and thermophotonic lock-in imaging for early detection of dental caries

Ashish Gupta, Ashkan Ojaghi, Elnaz Baradaran Shokouhi, Nima Tabatabaei, York Univ. (Canada)

The prevalence of caries among children and adult populations worldwide suggests that the current standard of care in Dentistry is not effective as restorations do not treat the underlying disease process but merely rebuild the tooth's shape. Therefore, Dentistry is undergoing a paradigm shift away from surgical treatment of caries to a model of early caries diagnosis and management (i.e., preventive Dentistry). However, existing clinical detection tools and techniques such as x-ray and visual/tactile inspection are not sensitive enough to detect early decay or monitor its progression. As such, in recent years, several promising optics-based methods have been developed for early detection of dental caries. The majority of these technologies rely on enhancement of light scattering in early carious lesion (e.g., optical coherent tomography or OCT) while few of them operate based on enhancement of light absorption in early caries (e.g., thermophotonic lock-in imaging or TPLI). In this paper, we present a systematic comparison between the detection performance of OCT and TPLI as two promising early dental caries detection imaging modalities which operate based on light scattering and light absorption, respectively. The comparative study is carried out through imaging of inception and progression of artificially-induced early proximal and occlusal caries in human teeth using an acidified gel. The comparison results of key performance parameters such as early caries detection threshold, maximum inspection depth, detection sensitivity, and imaging time will be presented and discussed.

10044-3, Session 1

Multimodal imaging of dental tissues and biomaterials

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Structural integrity and chemical composition were always considered to be the key factors in assessing the health of dental tissues. In this report, we supplement Raman spectroscopy and polarimetry imaging with mechanical characterization using Brillouin microspectroscopy [1]. We demonstrate for the first time multimodal imaging of dental tissues and related biomaterials [2].

References:

[1] Z. Meng, et al Advances in Optics and Photonics 8 (2), 300-327 (2016).

[2] Z. Meng et al, Advanced Materials (2016) Under Review.

10044-4, Session 1

A pre-clinical early caries detector based on optical coherence tomography and polarized Raman spectroscopy

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Early detection of incipient caries would allow dentists to provide more effective measures to delay or to reverse caries' progression at earlier stage. Such earlier intervention could lead to improved oral health for the patients and reduced burden to the health system. Previously, we have demonstrated that the combination of morphological and biochemical information furnished by optical coherence tomography (OCT) and polarized Raman spectroscopy (PRS), respectively, provided a unique tool for dental caries management. In this study we will report the first pre-clinical caries detection system that includes a hand-held probe with a size slightly larger than a tooth brush. This probe presents a novel platform combining both OCT and PRS optics in a very tight space ideal for clinical practice. OCT cross-sectional images of near-surface enamel morphology are obtained with miniaturized MEMS scanning device and are processed in real-time to identify culprit regions. These regions are sequentially analyzed with polarized Raman spectroscopy for further confirmation. PRS is performed using 830nm laser line and four detection channels in order to obtain polarized Raman spectroscopic data, i.e. depolarization ratio of the hydroxyapatite Raman band at ~960 cm-1. A detailed description of this hand-held caries detector and ex-vivo/in-vivo test results will be presented.

10044-5, Session 2

Characterization of human oral tissue based on quantitative analysis of optical coherence tomography images

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In this study, five types of tissues, human enamel, human cortical bone, human trabecular bone, muscular tissue, and fatty tissue were imaged ex vivo using optical coherence tomography (OCT). The specimens were prepared in blocks of 5 ? 5 ? 3 mm (width ? length ? height). The OCT imaging system was a swept source OCT system operating at wavelengths ranging between 1250 nm and 1360 nm with an average power of 18 mW and a scan rate of 50 to 100 kHz. The imaging probe was placed on top of a 2 ? 2 cm stabilizing device to maintain a standard distance from the samples. Ten image samples from each type of tissue were obtained. To acquire images with minimum inhomogeneity, imaging was performed multiple times at different points. Based on the observed texture differences between OCT images of soft and hard tissues, spatial and spectral features were quantitatively extracted from the OCT images. The Radon transform from angles of 0 deg to 90 deg was computed, averaged over all the angles, normalized to peak at unity, and then fitted with Gaussian function. The mean absolute values of the spatial frequency components of the OCT image was considered as a feature, where 2-D fast Fourier transform (FFT) was done to OCT images. These OCT features can reliably differentiate between a range of hard and soft tissues and could be extremely valuable in assisting dentists for in vivo evaluation of oral tissues and early detection of pathologic changes in tissues.

10044-6, Session 2

Assessing cavitation in artificial approximal dental lesions with near-IR imaging technologies

Jacob C. Simon, Cynthia L. Darling, Daniel Fried, Univ. of California, San Francisco (United States)

Bitewing radiography is still considered the state-of-the-art diagnostic technology for assessing cavitation within approximal carious dental lesions, even though radiographs cannot resolve cavitated surfaces but instead are used to measure lesion depth in order to predict cavitation. Clinicians need new technologies capable of determining whether approximal carious lesions have become cavitated because not all lesions progress to cavitation. Assessing lesion cavitation from near-infrared (NIR) imaging methods holds great potential due to the high transparency of enamel in the NIR region from ?=1300-1700-nm, which allows direct visualization and quantified measurements of enamel demineralization. The objective of this study was to measure the change in lesion appearance between non-cavitated and cavitated lesions in artificially generated lesions using NIR imaging modalities (two-dimensional) at ?=1300-nm and ?=1450-nm and cross-polarization optical coherence tomography (CP-OCT) (thee-dimensional) ?=1300-nm. Extracted human posterior teeth with sound proximal surfaces were chosen for this study and imaged before and after artificial lesions were made. A high speed dental hand piece was used to create artificial cavitated proximal lesions in sound samples and imaged. The cavitated artificial lesions where then filled with hydroxyapatite powder to simulate non-cavitated proximal lesions.

10044-7, Session 2

Near-infrared imaging of enamel hypomineralization due to developmental defects

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The increasing prevalence of mild hypomineralization due to developmental defects of enamel on tooth surfaces poses a challenge for caries detection and caries risk assessment and reliable methods need to be developed to discriminate such lesions from active caries lesions that need intervention. Previous studies have demonstrated that areas of hypomineralization are typically covered with a relatively thick surface layer of highly mineralized and transparent enamel similar to arrested lesions. Seventy-six extracted human teeth with mild to moderate degrees of suspicious fluorosis were imaged using near-infrared reflectance and transillumination. Enamel hypomineralization was clearly visible in both modalities. However, it was difficult to distinguish hypomineralization due to developmental defects from caries lesions with contrast measurements alone. The location of the lesion on tooth coronal surface (i.e. generalized vs. localized) seems to be the most important indicator for the presence of enamel hypomineralization due to developmental defects.

10044-8, Session 2

Assessing the dynamic biofilm removal of sulfonated phenolics using CP-OCT

Julia Nikrad, Karin Englund, Robert S. Jones, Univ. of Minnesota School of Dentistry (United States)

Examining the physical mechanisms related to biofilm removal of sulfonated phenolics (SP) is difficult using conventional microscopy techniques. A custom flow cell system integrated with a real time cross polarization optical coherence tomography system investigated the dynamic speed of biofilm removal when oral multi-species biofilms are exposed to SP under sheer stress. The Near infrared 1310-nm CP-OCT system non-destructively imaged fluid immersed oral biofilms at nearly 20 frames/s. This dynamic imaging was able to determine the cohesive and adhesion related disruption of SP on oral biofilms adhering to tooth like surfaces. For multi-species biofilms that are initially grown without the presence of sucrose, the disruption of biofilms on saliva coated hydroxyapatite (HA) is dominated as a adhesive failure at the HA-biofilm interface. For multi-species biofilms that are grown in the presence of sucrose, the disruption is dominated by cohesive disruption followed by adhesive failure. This novel CP-OCT flow cell assay has the potential to examine rapid interactions between anti-biofilm agents and tooth like surfaces.

10044-9, Session 3

Using of compact Nd:YAG laser operating at 1.06, 1.32, and 1.44 um for disinfection in restorative dentistry

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The aim of our study was to analyse the disinfection effect of Nd:YAG laser operating at 1.06, 1.32, and 1.44 um for patients with high concentration

of Streptococcus mutans in saliva (positive result in Saliva-check mutans test). The patients with higher concentration of Streptococcus mutans are at significantly higher caries risk. They have significantly more caries lesions (5.70) than patients who tested negative (2,70 caries lesions).

For the interaction the Nd:YAG laser system generated radiation at three switchable wavelengths with the maximum output energies 1.1, 0.6, and 0.3 J for wavelength 1.06, 1.32, and 1.44 μm, respectively, was used. For every patient the three saliva samples with high concentration of Streptococcus mutans were irradiated separately by the laser radiation with 3 various above mentioned wavelengths. The interaction energy densities were 99.7, 28.5, and 3.6 J/cm2 for 1.06, 1.32, and 1.44 μm wavelength, respectively.

The disinfection effect was evaluated in patient's saliva which was tested positive in Saliva-check mutans test. Our study proved that after the laser irradiation the Saliva-check test showed negative presence of Streptococcus mutans. The disinfection effect was confirmed for all used radiation wavelength. For 1.44 um this effect was reached with a smallest energy density.

10044-10, Session 3

Investigations on the potential of a novel diode pumped Er:YAG laser system at high mean laser power for hard tissue preparation in dentistry

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Several studies have shown the potential of the diode pumped Er:YAG laser for medical applications. Benefits are the efficient and precise ablation of both hard and soft tissue with variable thermal effects.

Aim of this study is the investigation of the ablation process on dentin and enamel using the diode pumped Er:YAG laser even at higher mean laser power and two different handpieces (free beam vs contact mode).

At first the laser light of the diode pumped Er:YAG laser (DPM20, Pantec Engineering AG)was coupled into a standard dental fiber delivery system (KEY3 laser, KaVo GmbH) with the two handpieces for cavity preparation. An appropriate experimental setup was realized including a computer controlled stepper unit with sample holder to move the dentin or enamel slides of extracted human teeth with a defined velocity while irradiation by various laser parameters. After irradiation the cuts were analyzed by light microscopy and laser scanning microscopy.

The results show a higher ablation rate on dentin and enamel using the contact handpiece compared to the focusing handpiece. Also the contact handpiece with water guidance directed to the ablation area allows irradiation at higher mean laser power without thermal damage. The results of the high speed camera observations explain the differences between the handpieces.

In conclusion these experiments with the diode pumped Er:YAG laser system demonstrate its ability for efficient and very fast cavity preparation.

10044-11, Session 3

Selective laser ablation of carious lesions using simultaneous scanned near-IR diode and CO2 lasers

Kenneth H. Chan, Daniel Fried, Univ. of California, San Francisco (United States)

Previous studies have shown that carious lesions can be imaged with high contrast using near-IR wavelengths coincident to water absorption, namely 1450-nm, without the interference of stains. Wavelengths coincident with even higher water absorption, specifically at 1900-nm, may yield higher lesion contrast and will be investigated. In this study, a point-to-point scanning system will be developed utilizing galvanometer scanners for acquiring reflected light at 1450 and 1900-nm from the tooth surface and lesion segmentation tools will be developed for lesion classification. This approach is advantageous since it does not require an expensive near-IR camera and we can detect even longer IR wavelengths unattainable with Si, Ge, or InGaAs near-IR cameras that may yield higher contrast. Furthermore, such imaging systems can be implemented with laser ablation systems using the same galvanometer scanners for the highly selective removal of carious lesions.

10044-12, Session 3

Developing laser-based therapy monitoring of early caries in pediatric dental settings

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Optical imaging modalities and therapy monitoring protocols are required for the emergence of non-surgical interventions for treating infections in teeth and remineralize the enamel. The current standard of x-ray imaging for caries detection is not highly sensitive, quantitative, and safe. Visual examination is subjective with the additional burden of meticulous record keeping, while haptic exploration is somewhat destructive and non-quantitative. The latter two are not viable options for interproximal caries.

We present preliminary results of multimodal laser-based imaging and fluorescence spectroscopy in a blinded study comparing two topical therapies of early interproximal caries in children. At baseline, with a spacer placed interproximally, the 405-nm excited red porphyrin fluorescence imaging with green autofluorescence rejection is measured and compared to a 12-month follow-up. 405-nm laser-induced fluorescence spectroscopy is also measured from the center of selected multimodal video imaging frames. These results are quantified and compared to the standard of care, visual examination and radiograph interpretation. At baseline (time zero) the fluorescence images and spectroscopic measures support the clinically determined degree of caries being present, lesion depth of less than half the enamel thickness.

By measuring the area of the porphyrin fluorescence regions at constant target-to-background ratio in matching images, a quantitative measure of the caries recovery process can be inferred. After two subjects completing the 12 month therapy monitoring study, quantitative analysis of fluorescence video frames demonstrate the challenges associated with optically monitoring non-surgical dental interventions over long periods of time in clinical practice.

10044-13, Session 4

A clinical handpiece for the automated ablation of dental composite using a CO2 laser coupled to a plume emission spectral feedback system

Andrew T. Jang, Univ. of California San Francisco (United States); Kenneth H. Chan, Daniel Fried, Univ. of California, San Francisco (United States)

With the advent of highly esthetic tooth matching dental materials, it becomes increasingly difficult for the operator to remove existing composite without excessive damage to the enamel or incomplete removal of the composite. Previous studies have utilized the changes in the excited plume emission spectra generated from laser ablation as a means to chemically differentiate composite from enamel. Using this novel detection system in combination with a galvanometer controlled infrared pulsed CO2 laser, we

have developed and tested, in vitro, a clinical handpiece for the automated selective removal of dental composite. The system uses dual photodiodes filtered to spectral ranges that best highlight the differences in the sodium and calcium emission line response (580nm and 600nm, respectively) of enamel and composite. Composite removal was confirmed and measured using Optical Coherence Tomography (OCT) in conjunction with 3D volumetric measuring tools developed with Matlab and Avizo. Future studies with this system include comparative clinical tests against traditional methods to ensure effective removal of composite in patients.

10044-14, Session 4

Laser Doppler pulp vitality measurements: simulation and measurements

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Frequently pulp vitality measurement is done in a dental practice by pressing a frozen cotton pellet on the tooth. This method is subjective, as the patient´s response is required, sometimes painful and has moderate sensitivity and specificity. Other methods, based on optical or electrical measurement have been published, but didn´t find wide spread application in the dental offices.

Laser Doppler measurement of the blood flow in the pulp could be an objective method to measure pulp vitality, but the influence of the gingival blood flow on the measurements is a concern. Therefore experiments and simulations were done to learn more about the gingival blood flow in relation to the pulpal blood flow and how to minimize the influence. First patient measurements were done to show the feasibility clinically.

Results:

Monte Carlo simulations and bench experiments simulating the blood flow in and around a tooth show that both basic configurations, transmission and reflection measurements are possible. Most favorable is a multi-point measurement with different distances from the gingiva. Preliminary sensitivity / specificity are promising and might allow an objective and painless measurement of tooth vitality.

10044-15, Session 4

Bond strength of a sealant resin to CO2 9.3 ?m short-pulse irradiated enamel

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The objective of this laboratory study was to evaluate whether irradiation of enamel with a CO2 9.3µm short-pulsed laser influences the shear bond strength of a sealant resin to enamel.

Bovine and human enamel samples were irradiated with a 9.3?m Carbon-dioxide laser (Solea, Convergent Dental, Inc., Natick, MA) with different laser energies known to ablate or render enamel caries resistant. Irradiation was performed “free-hand” or using a computerized motor driven stage. The total-etch system used was Scotchbond Universal etchant, the bonding agent Adper Single Bond Plus, and the sealant composite was Z250 Filtek supreme flowable (all 3M ESPE, St. Paul, MN). After 24 hours of storing in water a single plane shear-bond test was performed (UltraTester, Ultradent Products, Inc., South Jordan, UT).

Results:

All laser treated samples showed equal or higher bond strength than non-laser treated controls. For bovine enamel all laser test groups demonstrated increased shear-bond strength over the controls. Using a computerized motor driven stage irradiation did not result in superior bond strength over free-hand irradiation.

Enamel rendered caries resistant by CO2 9.3?m short-pulsed laser irradiation showed equal or significantly higher shear-bond strength to the sealant resin compared to non-laser irradiated enamel. Consequently, loss of sealants from CO2 9.3 ?m short-pulsed laser irradiated enamel is reduced. If enamel was laser ablated using higher laser energies before placing a sealant, the CO2 9.3?m laser cut surface again showed equivalent or superior bond strength to the flowable sealant.

10044-16, Session 4

Influence of multi-wavelength laser irradiation of enamel and dentin surfaces at 0.355, 2.94, and 9.4 um on surface morphology and acid resistance

Nai-Yuan N. Chang, Jacob C. Simon, Kenneth H. Chan, Jamison Jew, Robert C. Lee, William A. Fried, Heajin Cho, Cynthia L. Darling, Daniel Fried, Univ. of California, San Francisco (United States)

UV and IR lasers can be used to specifically target protein, water, and the mineral phase of dental hard tissues to produce varying changes in surface morphology. In this study, we irradiated enamel and dentin surfaces with various combinations of lasers operating at 0.355, 2.94, and 9.4 ?m, exposed those surfaces to topical fluoride, and subsequently evaluated the influence of these changes on acid resistance and permeability. Digital microscopy, polarized light microscopy, near-IR reflectance, fluorescence, surface dehydration rate measurements, and polarization sensitive optical coherence tomography were used to monitor changes in the samples overtime.

10044-17, Session PSun

Selective removal of natural caries lesions from tooth occlusal surfaces using a diode-pumped Er:YAG laser

Jamison Jew, Kenneth H. Chan, Daniel Fried, Cynthia L. Darling, Univ. of California, San Francisco (United States)

Selective removal of caries lesions with high precision is best accomplished using lasers operating at high pulse repetition rates utilizing small spot sizes. Conventional flash-lamp pumped Er:YAG lasers are poorly suited for this purpose, but new diode-pumped Er:YAG lasers have become available operating at high pulse repetition rates. The purpose of this study was to measure the selectivity of removal of natural caries lesions in tooth occlusal surfaces of extracted teeth using a 30 W diode-pumped Er:YAG laser operating with a pulse duration of 50-60-?s. Optical coherence tomography was used to measure the lesion volume prior to removal and measure the volume of tissue removed to assess selectivity.

10044-18, Session PSun

High-contrast reflectance imaging of composite restorations color-matched to tooth structure at 1000-2300-nm

William Fried, Univ. of California, San Francisco (United States); Jacob Simon, Univ of California San Francisco (United States); Cynthia L. Darling, Univ. of California, San Francisco (United States); Oanh Le, Univ of California San Francisco (United States); Daniel Fried, Univ. of California, San Francisco (United States)

One major advantage of composite restoration materials is that they can

be color matched to the tooth. However, this presents a challenge when composites fail and they need to be replaced. Dentists typically spend more time repairing and replacing composites than placing new restorations. We have shown in previous studies that high-contrast images of composite can be acquired in occlusal transmission mode at near-IR wavelengths coincident with higher water absorption. The purpose of this study was to determine if similar high-contrast images can be acquired in reflectance mode at longer wavelengths where water absorption is even higher. Extracted human teeth with existing composite restorations (n=16) were imaged at wavelengths from 1000-2300 using an extended range InGaAs camera. Our results indicate that NIR wavelengths longer than 1400-nm coincident with higher water absorption yield the highest contrast between dental composites and tooth structure in reflectance.

10044-19, Session PSun

Assessment of radicular dentin permeability after irradiation with CO2 laser and endodontic irrigation treatments using thermal imaging

Daniel Fried, Heajin Cho, Robert C. Lee, Kenneth H. Chan, Univ. of California, San Francisco (United States)

Previous studies have demonstrated that thermal imaging can be used to investigate changes in the surface morphology of dentinal surfaces. CO2 lasers have been shown to remove the smear layer and disinfect root canals. Moreover, CO2 lasers can be used to convert carbonated hydroxyapatite in enamel into a purer phase hydroxyapatite. The purpose of this study was to evaluate changes in the permeability of radicular dentin after CO2 laser irradiation and chemical treatments. Human molar specimens (n=12) were sectioned exposing the axial walls of the pulp chamber; walls were treated with either 10% NaOCl, 5% EDTA or a CO2 laser. The CO2 laser was operated at 9.4μm with a pulse duration of 26-μs, a pulse repetition rate of 300 Hz and a fluence of ~1 J/cm2. The samples were dehydrated using an air spray for 60 seconds and imaged using a thermal camera. The resulting surface morphological changes were assessed using 3D digital microscopy. The rate of dehydration was significantly altered after treatments with EDTA and CO2 laser ($P<0.05$). These results indicate that the surface modification due to CO2 laser treatment increases the permeability of radicular dentin.

10044-20, Session PSun

Synergistic effect of fluoride and laser radiation for the inhibition of the demineralization of dental enamel

Raymond Lee, Kenneth H. Chan, Jacob C. Simon, Daniel Fried, Univ. of California, San Francisco (United States)

Both laser irradiation and fluoride treatment alone are known to provide increased resistance to acid dissolution. CO2 lasers tuned to a wavelength of 9.3 μm can be used to efficiently convert the carbonated hydroxyapatite of enamel to a much more acid resistant purer phase hydroxyapatite (HAP). Further studies have shown that fluoride application to HAP yields fluoroapatite (FAP); which is an even more resistant against acid dissolution. Previous studies show that CO2 lasers and fluoride treatments interact synergistically to provide significantly higher protection than either method alone, but the mechanism of interaction has not been elucidated. We recently observed the formation of microcracks or a “crazed” zone in the irradiated region that is resistant to demineralization using high-resolution microscopy. The microcracks are formed due to the slight contraction of enamel due to transformation of carbonated hydroxyapatite to the more acid resistant pure phase hydroxyapatite (HAP) which has a smaller lattice. In this study, we test the hypothesis that these small cracks will provide greater adhesion for topical fluoride for greater protection against acid demineralization.

10044-21, Session PSun

Optical changes of dentin in the near-IR as a function of mineral content

Rhett Berg, Jacob C. Simon, Daniel Fried, Cynthia L. Darling, Univ. of California, San Francisco (United States)

The optical properties of human dentin can change markedly due to aging, friction from opposing teeth, and acute trauma, resulting in the formation of transparent or sclerotic dentin with increased mineral density. The objective of this study was to determine the optical attenuation coefficient of human dentin tissues with different mineral densities in the near-infrared (NIR) spectral regions from 1300-2200 nm using NIR transillumination and optical coherence tomography (OCT). N=50 dentin samples of varying opacities were obtained by sectioning whole extracted teeth into ≈150?m transverse sections at the cement-enamel junction and the apical root. Transillumination images were acquired with a NIR camera and attenuation measurements were acquired at various NIR wavelengths using a NIR sensitive photodiode. Samples were imaged with transverse microradiography (gold standard) in order to determine the mineral density of each sample.

10044-22, Session PSun

Rapid optical bacterial detection during endodontic treatment

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Bacteria present in the root canal (RC) space following a root canal treatment (RCT) are the main cause of persistent infections, resulting in treatment failure and the need for re-intervention or tooth extraction. Currently, there are no standardised methods in use to clinically detect bacterial presence within RC spaces. The use of paper point sampling and fluorescence staining was shown to be a rapid method, able to detect residual bacteria following treatment.

The study demonstrated that Calcein AM (CAM) proved to be a suitable dye for detecting vital bacteria within mature endodontic biofilms, with an improved sensitivity over CFU counting in a stressed biofilm model. Furthermore, in a clinical trial using primary RCTs, 53 infected teeth were sampled in-vivo and increased detection of vital cells was found when compared to CFU counting, highlighting the sensitivity of the technique in detecting low cell numbers.

Combining fluorescent staining and micro-spectroscopy with software-based spectral analysis, successful detection of vital cells from RCs was possible after 5 minutes of CAM incubation. Application of this technology during RCT has the potential to reduce persistent infections through vital cell detection and additional treatment. Furthermore, this technique could be applied to anti-microbial research and disinfection control in clinical settings.

10045-1, Session 1

Assessing corneal viscoelasticity after crosslinking at different IOP by noncontact OCE and a modified Lamb wave model

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UV-A Riboflavin collagen cross-linking (UV-CXL) is a promising treatment for keratoconus that stiffens mechanically degraded corneal tissue. On the other hand, the intraocular pressure (IOP) can also affect the measured cornea elasticity. However, the combined effects of CXL at different IOPs on the corneal biomechanical properties are not well understood, particularly in the whole eye-globe configuration. In this work, the feasibility of assessing the viscoelasticity of the porcine cornea before and after CXL at various IOPs was investigated by using a noncontact method of optical coherence elastography (OCE) and a modified Lamb wave model. The modified Lamb wave model was first verified by comparison with finite element modeling, and then utilized to analyze the OCE-measured data to quantify the viscoelasticity of porcine corneas in the whole eye-globe configuration before and after CXL treatment at various IOPs. The results show that the elasticity of the cornea increased after CXL and that corneal stiffness was linear as a function of IOP. At IOPs of 15, 20, 25, and 30 mmHg, the relative increase in Young's modulus after CXL was ~109%, ~86%, ~64%, and ~79%, respectively, while the shear viscosity decreased by ~86%, ~84%, ~83%, and ~81%. The modified Lamb wave model and OCE shows promise for quantifying corneal viscoelasticity, which could provide a basis for customized CXL therapies and disease detection.

10045-2, Session 1

Biomechanical properties of crystalline lens as a function of intraocular pressure assessed noninvasively by optical coherence elastography

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The crystalline lens plays important role in forming vision. Various ocular diseases, such as cataract and presbyopia, can alter the biomechanical properties of the lens, subsequently degrading visual acuity. Additionally, ocular diseases such as glaucoma and uveitis can lead to an elevation of intraocular pressure (IOP). Previous research has implied a link between elevated IOP and lens pathology. However, the relationship between IOP elevation and biomechanical properties of the crystalline lens has not been directly studied yet. The location of the crystalline lens inside the eye makes it very challenging to measure its mechanical properties in vivo or in situ. In this work, we utilized a co-aligned acoustic radiation force and phase-sensitive optical coherence elastography (OCE) system to study the biomechanical properties of the in situ porcine lens as a function of IOP. The OCE results demonstrate that the stiffness of the lens increased with IOP and the changes in the elasticity of the lens were reversible in the short term.

10045-3, Session 1

Assessing the mechanical anisotropy and hysteresis while cycling IOP of porcine corneas before and after CXL by noncontact optical coherence elastography

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Diseases such as keratoconus can alter the orientation of collagen fibrils in the cornea. Moreover, therapeutic interventions such as UV-A/riboflavin corneal collagen crosslinking (CXL) can alter the collagen fibril arrangement. Therefore, the anisotropic characteristics of the cornea can provide vital information about tissue integrity. In this work, we utilize noncontact elastic wave imaging optical coherence elastography (EWI-OCE) to assess the elastic anisotropy and hysteresis of in situ porcine corneas as various intraocular pressures (IOP). In addition, we evaluated the effects of CXL on the mechanical anisotropy and hysteresis. OCE measurements were made at stepped meridional angles, and a sliding window algorithm spatially mapped the elasticity. A modified planar anisotropy coefficient was utilized to quantify the elastic anisotropy of the corneas. The results show that the stiffness and elastic anisotropy of the corneas were significantly affected by CXL and IOP ($P<0.001$), but the hysteresis was not significant ($P<0.05$). Moreover, the changes in elasticity due to CXL were angle-dependent ($P<0.005$). However, the changes in mechanical anisotropy from CXL were not angle-dependent ($P>0.05$).

10045-4, Session 1

Effect of increased intraocular pressure on the pulsatile response in the posterior rat eye

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Fundus pulsations depend on the biomechanics of the eye ball and can be linked to choroidal and retinal perfusion, which play an important role in various ocular diseases such as glaucoma. To investigate this interaction, the intraocular pressure (IOP) in non-pigmented rats was experimentally increased until retinal perfusion changed substantially. High speed retinal OCT imaging was performed at different IOP levels. The ocular pulse and OCT angiography were simultaneously captured by slowly scanning an area of 1.5 mm ? 1.5 mm around the optic nerve head. The axial velocity between the retina and the chorioscleral complex was determined by calculating the phase differences between consecutive B-scans and dividing the images into two slabs. We were able to identify the ocular pulse by analyzing the axial velocities of the contramotion between the investigated regions. The power of the velocity spectrum was used to correlate the ocular pulse with different IOP levels and the retinal perfusion using OCT angiography.

10045-5, Session 2

Comparison of continuous versus pulsed photodynamic antimicrobial therapy for inhibition of fungal keratitis isolates in vitro

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Fungal keratitis can lead to pain and impaired vision. Current treatment options include antifungal agents and therapeutic penetrating keratoplasty. An emerging option for the management of keratitis is photodynamic antimicrobial therapy (PDAT) which uses a photosensitizer rose bengal activated with green light. Utilizing a pulsed irradiation, rather than the standard continuous irradiation may have a similar antimicrobial effect with less total energy. This study is to compare pulsed and continuous rose bengal mediated PDAT for inhibition of six fungal isolates on agar plates: Fusarium solani, Fusarium keratoplasticum, Aspergillus fumigatus, Candida albicans, Paecilomyces variotti, and Pseudoallescheria boydii. Isolates were mixed with 0.1% rose bengal and exposed to three irradiation conditions: (1) 30-minute continuous (10.8J/cm2), (2) 15-minute continuous (5.4J/cm2), (3) 30-minute pulsed (5.4J/cm2). Plates were photographed at 72 hours and analyzed with custom software. At 72 hours, 30-minute continuous rose bengal mediated PDAT inhibited all six fungal species. Fungal inhibition was analogous between 30-minute continuous and 30-minute pulsed test groups, with the exception of A. fumigatus. The 15-minute continuous irradiation was less effective when compared to both 30-minute continuous and 30-minute pulsed groups. These in vitro results demonstrate the potential strength of pulsed rose bengal mediated PDAT as an adjunct treatment modality for fungal keratitis.

10045-6, Session 2

Phase-resolved OCT measurement on tissue denaturation toward real-time monitoring of retinal laser coagulation

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Retinal laser coagulation is used to treat some posterior eye diseases.

However, it causes damage to tissues surrounding target location.

In this study, we present optical path length change measurement by using phase-resolved OCT to detect tissue alterations during laser illumination.

Tissue denaturation may cause morphological alteration and refractive index change.

We measure the optical path length change during the illumination on ex vivo porcine retinas.

Spectral-domain OCT system was combined with coagulation laser (532 nm wavelength) in previous study.

For ex vivo porcine eyes, coagulation laser was illuminated.

OCT M-mode scan near the center of coagulation laser illumination was applied simultaneously.

Several different laser illumination conditions (power and duration) were applied at different locations on the retina.

Three dimensional OCT volumes were acquired before and after laser illumination to confirm whether laser lesion was generated or not.

To detect instantaneous local tissue alteration, local optical path length (LOPL) change is measured by using phase of complex OCT signal.

From this LOPL change, a metric is introduced to represent a net tissue alteration.

Multiple logistic regression analysis was applied to evaluate the metric.

The outcome is visibility of laser lesion after laser illumination.

As explanatory variables, the metric, laser power, and laser duration are used.

Only the metric is statistically significant to reject the null-hypothesis (not explain the outcomes).

Hence, the metric may can be used as an indicator of laser lesion generation.

This approach may be suitable to monitor the generation of laser lesion.

10045-7, Session 2

Tissue response to micropulse modulation in retinal laser therapy

Jenny Wang, Yi Quan, Roopa Dalal, Daniel V. Palanker, Stanford Univ. (United States)

Micropulse modulation in retinal laser therapy was intended to confine tissue heating around the light-absorbing layers, such as RPE and choroid, while the transparent retina is heated less as a result of slow heat diffusion. Current implementations use micropulses of 100-300?s at 500Hz, with overall pulse envelope of 100-300ms. The effect of such modulation compared to continuous-wave (CW) is not well characterized and misleading comparisons are made in the literature between exposures of different average power or overall duration. In this study, we modeled and measured the retinal tissue response to pulse trains with duty cycles from 4% (80?s pulse at 500Hz) to CW at overall envelope of 200ms and 20ms. Three thresholds of tissue response were measured in Dutch-belted rabbits: immediate (<3s after laser delivery) and delayed (1-5min) ophthalmoscopic visibility of lesions corresponding to photoreceptor damage, as well as fluorescein angiography visibility indicating RPE damage. Both the model and experimental results show that tissue response to micropulse modulation with long pulse envelope (200ms) is not significantly different from CW exposures at the same average power and duration. Heat confinement is improved with lower duty cycle (2%) and shorter pulse envelope (20ms), however further decrease in exposure duration raises the temperature dangerously close to vaporization. Pulse modulation cannot improve the therapeutic range of non-damaging thermal therapy since it is defined by the Arrhenius integral, regardless of the time course of hyperthermia. However, it does allow greater thermal stress to the RPE and underlying choroid while avoiding damage to neural retina.

10045-8, Session 2

Photo-mediated ultrasonic antivascular therapy: a novel method of selectively treating neovascularization

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Retinal and choroidal neovascularization play a pivotal role in the leading causes of blindness including macular degeneration and diabetic retinopathy (DR). Current therapy by focal laser photocoagulation can damage the normal surrounding cells, such as the photoreceptor inner and outer segments which are adjacent to the retinal pigment epithelium (RPE), due to the use of high laser energy and millisecond pulse duration. Therapies with pharmaceutical agents involve systemic administration of

drugs, which can cause adverse effects and patients may become drug-resistant.

We have developed a noninvasive photo-mediated ultrasound therapy (PUT) technique as a localized antivascular method, and applied it to remove micro blood vessels in the retina. PUT takes advantage of the high native optical contrast among biological tissues, and has the unique capability to self-target microvessels without causing unwanted damages to the surrounding tissues. This technique promotes cavitation activity in blood vessels by synergistically applying nanosecond laser pulses and ultrasound bursts. Through the interaction between cavitation and blood vessel wall, blood clots in microvessels and vasoconstriction can be induced. As a result, microvessels can be occluded. In comparison with other techniques that involves cavitation, both laser and ultrasound energy needed in PUT is significantly lower, and hence improves the safety in therapy.

10045-9, Session 2

Vortex-beams for precise and gentle dissection in refractive corneal surgery

Alfred Vogel, Sebastian Freidank, Norbert Linz, Univ. zu Lübeck (Germany)

In LASIK, an incision parallel to the corneal surface creates a thin 'flap', and excimer laser ablation of stromal material corrects the refractive error. In SMILE, two intrastromal incisions produce a lenticule that is removed with forceps through small side cuts. For dissection, laser pulses are focused in a raster pattern into the corneal stroma and plasma-induced microexplosions generate cavitation bubbles that cleave the lamellae. Cutting precision is compromised by the large focus length associated with commonly used IR wavelengths and moderate NAs. Based on investigations of the cutting dynamics, we present a novel approach for corneal dissection using ring foci from vortex beams, and demonstrate possible improvements by using shorter wavelengths.

Laser-induced bubble formation in corneal stroma is investigated by high-speed photography at 1-50 million frames/s. Incident and absorbed laser energy needed for easy removal of flaps created in porcine corneas are determined for Gaussian and vortex beams with pulse durations from 300 fs to 9 ps for IR wavelengths and 1 ps to 850 ps for UVA wavelengths. The cutting quality is documented by scanning electron microscopy.

Vortex beams produce a short, donut-shaped focus allowing for efficient and precise dissection along the corneal lamellae. This dramatically reduces the absorbed energy needed for cutting, diminishes bubble formation in the cutting plane as well as mechanical side effects, and leads to a smoother dissection. These results are of special interest for improving SMILE, where the refractive outcome largely relies on the precision of lenticule dissection.

10045-76, Session 3

Clinical implementation of fs cataract surgery, needs for further technology? *(Keynote Presentation)*

William W. Culbertson, Bascom Palmer Eye Institute (United States)

No Abstract Available

10045-10, Session 4

Methods for non-surgical cancer nano-theranostics of ocular tumors in the mouse eye

Mayank Goswami, Xinlei Wang, Pengfei Zhang, Wenwu Xiao, Kit S. Lam, Edward N. Pugh Jr., Robert J. Zawadzki, Univ. of California, Davis (United States)

We will present our results of evaluating the feasibility of using the mouse eye as a window for non-invasive, long-term, optical investigation of xenograft models, using multimodal, cellular-resolution ocular imaging. As an "approachable part of the brain", the retina allows examination of such issues as drug delivery across the blood retinal barrier (BRB) and blood brain barrier (BBB). Our custom-built wide-field SLO/OCT provided repeatable in vivo imaging over many weeks, allowing quantitative tracking of tumor growth, the delivery of theranostic nanoparticles, and the measurement of tumor microenvironment responses. Additionally, we were able to specifically control the spatial extent of light activated photodynamic therapy (PDT) and photothermal therapy (PTT) via efficient free radical and heat generation at the tumor site, respectively.

10045-11, Session 4

Polarisation-sensitive visible light OCT for mouse retinal imaging

Danielle J. Harper, Marco Augustin, Antonia Lichtenegger, Carlos Reyes, Pablo Eugui, Stanislava Fialová, Michael Pircher, Christoph K. Hitzenberger, Bernhard Baumann, Medizinische Univ. Wien (Austria)

Non-invasive in-vivo imaging techniques such as optical coherence tomography (OCT) are valuable as they allow longitudinal studies of the retina over time. Already two well-established methods, both polarization-sensitive (PS) OCT and visible light OCT (vis-OCT) have proven themselves independently as useful tools in ophthalmic imaging. In this work we combine these features and present a new PS-OCT system operating in the spectral domain over the visible light range (430-700 nm). The system has been specifically designed for the imaging of the mouse retina. With such a broad bandwidth and low central wavelength, an axial resolution of 1.14 μm in air and 0.83 μm in tissue has been achieved. Preliminary images acquired by this system show the retina of a healthy mouse model. In these images, features such as the optic nerve head and the retinal pigment epithelium can be clearly distinguished, and the various layers which make up the retina can be seen. We demonstrate for the first time a system which has the potential to analyze visible light spectroscopic image data as well as reflectivity and PS-OCT contrast images. Such images suggest that this new system may be a promising tool for the future of ophthalmic imaging.

10045-12, Session 4

In vivo photothermal optical coherence tomography of targeted gold nanorods in the mouse eye

Maryse Lapierre-Landry, Andrew Y. Gordon, John S. Penn, Vanderbilt Univ. (United States); Melissa C. Skala, Morgridge Institute for Research (United States)

Optical coherence tomography (OCT) has become standard in retinal imaging at the pre-clinical and clinical level by allowing non-invasive, three-dimensional imaging of the tissue structure. However, OCT lacks specificity to contrast agents that could be used for in vivo molecular imaging. We have performed in vivo photothermal optical coherence tomography (PT-OCT) of targeted gold nanorods in the mouse retina after the mice were injected systemically with the contrast agent. To our knowledge, we are the first to perform PT-OCT in the eye and image targeted gold nanorods with this technology. As a model of age-related wet macular degeneration, lesions were induced by laser photocoagulation in each mouse retina (n=12 eyes). Untargeted and targeted (anti-mouse CD102 antibody, labeling neovasculature) gold nanorods (peak absorption ?=750nm) were injected intravenously by tail-vein injection five days after lesion induction, and imaged the same day with PT-OCT. Our instrument is a spectral domain OCT system (?=860nm) with a Titanium:Sapphire laser (?=750nm) added to the beam path using a 50:50 coupler to heat the gold nanorods. We acquired

PT-OCT volumes of one lesion per mouse eye. There was a significant increase in photothermal intensity per unit area of the lesion in the targeted gold nanorods group versus the saline control group and the untargeted gold nanorods group. This experiment demonstrates the feasibility of PT-OCT to image the distribution of molecular contrast agents in the mouse retina, including in highly scattering lesions. In the future we will use this method to identify new biomarkers linked with retinal disease.

10045-13, Session 4

Light induced increases of photoreceptor layer reflectance in response to rhodopsin bleaching in mice measured in vivo with optical coherence tomography

Pengfei Zhang, Univ. of California, Davis (United States); Mayank Goswami, Univ of California Davis (United States); Edward N. Pugh Jr., Robert J. Zawadzki, Univ. of California, Davis (United States)

We have recently reported observations of light-induced broadband fundus reflectance changes in two most commonly used strains of laboratory mice, C57Bl/6J (pigmented) and Balb/c (un- pigmented albino). The action spectrum of the reflectance increase corresponded to the absorption spectrum of mouse rhodopsin in situ. Spectral changes in mouse fundus reflectivity were calculated from measurements made by broadband spectrometer, interfaced with our mouse retinal SLO system, obtained before and after bleaching. This results were fitted with a model of mouse fundus reflectance, quantifying contributions from loss of rhodopsin absorption with bleaching, absorption by oxygenated hemoglobin (HbO2) in the choroid (Balb/c), and absorption by melanin (C57Bl/6J) additionally both mouse strains exhibited light-induced broadband reflectance changes explained as bleaching-induced reflectivity increases at photoreceptor inner segment/outer segment (IS/OS) junctions and OS tips. Here we present results investigating the kinetics of the increases in reflectivity with Optical Coherence Tomography operating in a 780-950 nm band.

10045-14, Session 4

Study on choroidal neovascularization with anti-VEGF treatment in the mouse retina using optical coherence tomography angiography

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To understand the pathogenesis of ophthalmic disease, utilizing small animal models such as mouse is necessary because of their ease of maintenance and availability. For identifying pathophysiology and drug development of retinal diseases in mouse model, optical coherence tomography angiography (OCTA) is promising imaging modality visualizing not only microstructure but also microvasculature. In this study, we serially imaged 3D structure and angiography of laser-induced choroidal neovascularization (CNV) in the mouse retina with/without anti-VEGF treatment. Also, the volume changes of CNV and avascular region in choroid layer are measured for identifying effects of anti-VEGF.

A lab-built high-speed OCTA prototype using the wavelength-swept laser centered at 1040 nm with 230 kHz A-scan rate acquired 3-D volumetric data consisted of 1024 x 1024 x 3 A-scans. The OCTA scanned 1.7 mm x 1.7 mm area around ONH. For obtaining angiography, amplitude decorrelation from 3 consecutive B-scans at each position was generated. Seven days after the laser photocoagulation at mouse retina for generation of the laser-induced CNV, intravitreal administration of Fc and VEGF-Trap was given in the therapeutic arm. The OCTA were performed at 6, 14, 21 and 35 days after laser photocoagulation. Vasculatures of inner retina, outer retina and choroid layers were separately visualized after RPE flattening and layer segmentation. To investigate therapeutic effects of anti-VEGF treatment, the relative area and volume of CNV in outer retina layer is measured. Also, total volume of avascular zone surrounding the laser injury site in choroid layer is also analyzed.

10045-15, Session 4

Quantification of rat retinal and choroidal blood plasma kinetics, volume, and flow in vivo using dynamic contrast optical coherence tomography

Conrad W. Merkle, Vivek J. Srinivasan, Univ. of California, Davis (United States)

Blood flow patterns and kinetics in the choriocapillaris are poorly understood owing to a lack of quantitative ophthalmic imaging techniques for studying microvascular flow in the eye. Compared with the proximal retinal vasculature, the more distal choroidal vasculature is relatively more challenging to probe. Magnetic Resonance Imaging and Doppler Ultrasound can assess the retina and choroid, but do not resolve the finer layers or microvasculature. While Optical Coherence Tomography (OCT) angiography produces high-quality choroidal images, attempts at quantification through Doppler-based methods have had mixed success.

Here, we use a new technique called Dynamic Contrast OCT (DyC-OCT), which tracks the passage of an intravascular scattering contrast agent, to reveal laminar blood flow patterns in the retina and choroid in vivo. While conceptually similar to fluorescence angiography, DyC-OCT has the substantial benefit of depth resolution, which enables separation of retinal and choroidal microvasculature. The scattering contrast agent enables improved angiography of both macro- and microvasculature in the retina and choroid. Blood plasma transit times are measured in individual vessels, while flow and volume are quantified for each of the microvascular layers. As expected, the choriocapillaris had the highest volume and flow. Blood flow rates were estimated with an average retinal blood flow of 9.1 ± 4.3 ?L/min and an average choroidal blood flow of 40 ± 18.3 ?L/min in the rat eye. These rates are consistent with previous literature. DyC-OCT affords a new perspective on the poorly understood choriocapillaris blood flow and kinetics and may be useful for studying outer retinal diseases.

10045-16, Session 4

Visually evoked changes in the rat retinal blood flow measured with Doppler optical coherence tomography

Bingyao Tan, Erik Mason, Ben MacLellan, Kostadinka Bizheva, Univ. of Waterloo (Canada)

Visually evoked changes of retinal blood flow can serve as an important research tool to investigate eye disease such as glaucoma and diabetic retinopathy. In this study we used a combined, research-grade, high-resolution Doppler OCT+ERG system to study changes in the retinal blood flow (RBF) and retinal neuronal activity in response to visual stimuli of different intensities, durations and type (flicker vs single flash). Specifically, we used white light stimuli of 10 ms and 200 ms single flash, 1s and 2s for flickers stimuli of 20% duty cycle. The study was conducted in-vivo in pigmented rats. Both single flash (SF) and flicker stimuli caused increase in the RBF. The 10 ms SF stimulus did not generate any consistent measurable response, while the 200 ms SF of the same intensity generated ~4% change

in the RBF peaking at ~1.5 s after the stimulus onset. Single flash stimuli introduced ~2x smaller change in RBF and ~30% earlier RBF peak response compared to flicker stimuli of the same intensity and duration. Doubling the intensity of SF or flicker stimuli increased the RBF peak magnitude by ~1.5x. Shortening the flicker stimulus duration by 2x increased the RBF recovery rate by 2x, however, had no effect on the rate of RBF change from baseline to peak.

10045-17, Session 4

Automated feature extraction for retinal vascular biometry in zebrafish using OCT angiography

Ivan Bozic, Vanderbilt Univ. (United States); Rose DiCicco, Alex Yuan, The Cleveland Clinic (United States); Yuankai K. Tao, Vanderbilt Univ. (United States)

Zebrafish have been identified as an ideal model for angiogenesis because of anatomical and functional similarities with other vertebrates. The scale and complexity of zebrafish assays are limited by the need to manually treat and serially screen animals, and recent technological advances have focused on automation and improving throughput. Here, we use optical coherence tomography (OCT) and OCT angiography (OCT-A) to perform noninvasive, in vivo imaging of retinal vasculature in zebrafish. OCT-A summed voxel projections were low pass filtered and skeletonized to create an en face vascular map prior to connectivity analysis. Vascular segmentation was referenced to the optic nerve head (ONH), which was identified by automatically segmenting the retinal pigment epithelium boundary on the OCT structural volume. The first vessel branch generation was identified as skeleton segments with branch points closest to the ONH, and subsequent generations were found iteratively by expanding the search space outwards from the ONH. Biometric parameters, including length, curvature, and branch angle of each vessel segment were calculated and grouped by branch generation. Despite manual handling and alignment of each animal over multiple time points, we observe distinct qualitative patterns that enable unique identification of each eye from individual animals. We believe this OCT-based retinal biometry method can be applied for automated animal identification and handling in high-throughput organism-level pharmacological assays and genetic screens. In addition, these extracted features may enable high-resolution quantification of longitudinal vascular changes as a method for studying zebrafish models of retinal neovascularization and vascular remodeling.

10045-18, Session 5

Changes to the cellular structure of corneal epithelium in keratoconic subjects imaged in-vivo with sub-micrometer axial resolution OCT

Kostadinka Bizheva, Bingyao Tan, Erik Mason, Kirsten Carter, Lacey Haines, Luigina Sorbara, Univ. of Waterloo (Canada)

Keratoconus causes progressive morphological changes in the corneal epithelium (EPI), Bowman's membrane (BM) and anterior stroma. However, it is still not well understood if KC originates in the corneal epithelium and propagates to the anterior stroma through disruptions of the BM, or vice versa. In this study we used a sub-micrometer axial resolution OCT system to image in-vivo the cellular structure of the EPI layer and the fibrous structure of the BM and the anterior stroma in mild to advanced keratoconics, as well as healthy subjects. The imaging study was approved by the University of Waterloo Human Research Ethics Committee. The OCT system operates in the 800 nm spectral region at 34 kHz image acquisition rate and provides 0.95 um axial and < 2 um lateral resolution in corneal tissue, which is sufficient to visualize the cellular structure of the corneal epithelium and the fibrous structure of the BM. In some subjects, localized thinning and thickening of the EPI layer was observed, while there was no visible damage to the BM or anterior stroma. In other subjects, localized breakage of the stromal collagen fibrils was observed with no significant morphological changes of the corneal EPI.

10045-19, Session 5

Characterization of the lamellar rearrangement induced by cross-linking treatment in keratoconic corneal samples imaged by SHG microscopy

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Keratoconus is an eye disorder that features a reduced stiffness of the cornea and its consequent pathological deformation. Cross-Linking (CXL) treatment has proven useful in hindering the progression of keratoconus, offering a minimally-invasive alternative to corneal surgical transplantation. In this study, the biomechanical characteristics of a human keratoconic cornea were examined in vivo soon before keratoplasty, and the morphological alterations of the collagen scaffold in the same cornea were examined ex vivo by means of Second-Harmonic Generation (SHG) microscopy. A healthy cornea and a CXL-treated keratoconus were compared. In particular, the lamellar organization in the three corneal samples was characterized in different stromal layers, by detecting both forward- and backward-scattered SHG signal and then considering the forward/backward (F/B) ratio as organization parameter. The F/B SHG ratio was used to characterize the morphological organization of collagen lamellae at different stromal levels, finding an increased disorder at the level of Bowman's membrane, opposed to a more regular organization within deeper stromal layers in all the examined samples. The organization of collagen lamellae in CXL-treated keratoconic samples was close to the healthy cornea ones, demonstrating that the CXL is able to rearrange the collagen scaffold and partially recover the properties of a healthy cornea. The results obtained with the presented analysis are in agreement with previous results obtained in studies aimed at monitoring the organization of fibrillar collagen using F/B SHG ratio. In conclusion, the proposed method might be useful for diagnosing keratoconus and to monitor the effects of the CXL treatment.

10045-20, Session 5

Ultrahigh resolution imaging of cellular dynamics in explanted retinas with dynamic full-field OCT

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Full-Field Optical Coherence Tomography (FF-OCT) reveals submicrometric morphological details in retinal explants without the use of contrast agents. Notably, in the nerve fiber and ganglion cell layers, FF-OCT images reveal nerve fibers bundles, single axons, capillaries and even some ganglion cell bodies.

Dynamic FF-OCT (D-FF-OCT) takes advantage of the temporal evolution of the local FF-OCT signal to reveal a movement-dependent contrast inside tissues. Notably, the D-FF-OCT signal depends on cellular motility and membrane fluctuations. Compared to regular FF-OCT images, the signal from stationary structures such as nerve fibers is reduced, and contrast inside cells is enhanced, revealing many more cells, as well as the position of nuclei, and cell metabolism.

We used a multimodal D-FF-OCT and fluorescence microscope to compare and identify the structures observed in both FF-OCT and D-FF-OCT.

In the ganglion cell and inner nuclear layers in both macaque and mouse, two different cell sizes could be measured, which correlated well with ganglion and amacrine cell diameters found in the literature for these two species. We could also detect cell bodies of the photoreceptors in the outer nuclear layer. To our knowledge, this is the first time that an OCT technique can reveal these cell bodies.

Finally, to investigate post-mortem tissue changes, time series were acquired over periods of 24 hours and cell contrast was plotted in time to monitor the decrease in intracellular activity over time.

It is anticipated that dynamic FF-OCT may be used to non-invasively monitor viability and functional changes in the retina.

10045-21, Session 5

Imaging of single retinal ganglion cell with differential interference contrast microscopy

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Glaucoma is a progressive optic neuropathy, characterized by the selective loss of retinal ganglion cells (RGCs). Therefore, monitoring the change of number or morphology of RGC is essential for the early detection as well as investigation of pathophysiology of glaucoma. Since RGC layer is transparent and hyporeflective, the direct optical visualization of RGCs has not been successful so far. Therefore, glaucoma evaluation mostly depends on indirect diagnostic methods such as the evaluation of optic disc morphology or retinal nerve fiber layer thickness measurement by optical coherence tomography.

We have previously demonstrated single photoreceptor cell imaging with differential interference contrast (DIC) microscopy. Herein, we successfully visualized single RGC using DIC microscopy. Since RGC layer is much less reflective than photoreceptor layer, various techniques including the control of light wavelength and bandwidth using a tunable band pass filter were adopted to reduce the chromatic aberration in z-axis for higher and clearer resolution. To verify that the imaged cells were the RGCs, the flat-mounted retina of Sprague-Dawley rat, in which the RGCs were retrogradely labeled with fluorescence, was observed by both fluorescence and DIC microscopies for direct comparison. We have confirmed that the cell images obtained by fluorescence microscopy were perfectly matched with cell images by DIC microscopy.

As conclusion, we have visualized single RGC with DIC microscopy, and confirmed with fluorescence microscopy.

10045-22, Session 5

Evaluation of intraretinal migration of retinal pigment epithelial cells with Jones matrix optical coherence tomography

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We evaluated intraretinal RPE migrations in AMD using polarimetric methods. Depolarized light images were computed using a PS-SLO at 780 nm. For PS-OCT imaging, we used multifunctional Jones-matrix OCT. This OCT system was based on swept-source technology, and operated at an axial scan speed of 100,000 A-scans/s, using a swept-source laser at a central wavelength of 1,048 nm. To evaluate the distribution of depolarization or polarization scramble in OCT B-scan images, we calculated degree of polarization uniformity (DOPU) with noise correction (Makita DOPU; M-DOPU). Each polarimetry image was compared with auto-fluorescence image at 500 nm (SW-AF) and 800 nm (NIR-AF). Intraretinal RPE migration was defined by the presence of depolarization at intraretinal hyper-reflective foci, and hyper-AF in both NIR-AF and SW-AF images at corresponding location. We evaluated 155 eyes with AMD; including 13 eyes with early to intermittent AMD, 13 eyes with drusenoid PED, 25 eyes with serous PED, 22 eyes of remission stage of exudative AMD, 69 eyes of end-stage of AMD with subretinal fibrosis, and 13 eyes of dry AMD with geographic atrophy. RPE migrations were detected in 59 of 155 eyes (38%). RPE migrations were observed in drusenoid PED and serous PED with significantly higher frequency than other groups ($P < 0.02$ and $P < 0.0001$ respectively). Similarities could be confirmed among en-face projection images of minimum M-DOPU, depolarized light images and NIR-AF images. Multimodal imaging including polarimetric imaging and AF imaging is an effective tool to understand the RPE changes in macular disease.

10045-23, Session 5

Impact of anatomical parameters on optical coherence tomography retinal nerve fiber layer thickness abnormality patterns

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Purpose: To evaluate the effect of four anatomical parameters (angle between major superior and inferior temporal retinal arteries [inter-artery angle, IAA], optic disc [OD] torsion, retinal curvature, and central retinal

vessel trunk entry point location [CRVTL]) on RNFLT abnormality marks by OCT machines.

Methods: Cirrus OCT circumpapillary RNFLT measurements and Humphrey visual fields (HVF 24-2) of 421 patients from a large glaucoma clinic were included. Ellipses were fitted to the OD borders. Ellipse rotation relative to the vertical axis defined OD torsion. CRVTL was manually marked on the horizontal axis of the ellipse on the OCT fundus image. IAA was calculated between manually marked retinal artery locations at the 1.73mm radius around OD. Retinal curvature was determined by the inner limiting membrane on the horizontal B-scan closest to the OD center. For each location on the circumpapillary scanning area, logistic regression was used to determine if each of the four parameters had a significant impact on RNFLT abnormality marks independent of disease severity. The results are presented on spatial maps of the entire scanning area.

Results: Variations in IAA significantly influenced abnormality marks on 38.8% of the total scanning area, followed by CRVTL (19.2%) and retinal curvature (18.7%). The effect of OD torsion was negligible (<1%).

Conclusions: A natural variation in IAA, retinal curvature, and CRVTL can affect OCT abnormality ratings, which may bias clinical diagnosis. Our spatial maps may help OCT manufacturers to introduce location specific norms to ensure that abnormality marks indicate ocular disease instead of variations in eye anatomy.

10045-24, Session 5

Evaluation of filtration surgery outcome using multi contrast anterior eye segment jones matrix optical coherence tomography

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Glaucoma is the second leading cause of blindness in the world. Filtration surgeries (trabeculectomy and ExPress Shunt) are the common procedure for medically uncontrollable glaucoma. Evaluating and predicting bleb function could help better understand and improve the successful outcome of the surgery. Multicontrast Jones matrix OCT (JM-OCT) can provide birefringence, vasculature, and structural scattering information along the depth in a single scan.

In this paper, a depth resolved evaluation of bleb function is demonstrated by using vasculature and birefringence contrast obtained by JM-OCT. 18 eyes of 15 subjects who had undergone filtration surgery were involved in the study. Descriptive as well as quantitative evaluations of the blebs are performed. Birefringence tomography is obtained using maximum a posteriori birefringence estimation and Vasculature tomography obtained using intensity variance analysis of JM-OCT signals. Fibrosis Score and Vascular index are the metrics used to quantitatively describe the birefringence and vasculature contrast seen along three depth zones of the bleb tissue. For descriptive case series, color photograph and the composite images of birefringence and vasculature for three depth zones are shown. The performance of these metrics were analyzed for each depth zones to understand the discriminatory power to assess the bad blebs.

Fibrosis score obtained for each zonal depth show good discriminatory ability to distinguish bad bleb from the good. Grading based on vasculature index was compared with that of the color photograph and the vasculature seen in the deeper zone. A high speed JM-OCT could provide a full quantitative analysis of vasculature for better understanding angiogenesis and fibrosis.

10045-25, Session 5

Further analysis of clinical feasibility of OCT-based glaucoma diagnosis with PIMD

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No Abstract Available

10045-26, Session 5

ONH study based on the multiple optical and biometric parameters

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Glaucoma is a group of eye diseases which results in optic nerve damage and vision loss. Optical coherence tomography (OCT) has been widely used to investigate geometric risk factor of glaucoma. However, material properties of ONH are also important to understand intra-ocular pressure related stress.

We developed Jones-matrix based multifunctional posterior eye OCT (JM-OCT), which uses 1-μm band swept-source with a 100-kHz A-line rate. It provides three different optical properties, attenuation coefficient (AC), local birefringence (LB), and optical coherence angiography (OCA). We investigated the utility those properties for the investigation of normal ONH cases. 3 mm ? 3 mm area around ONH was scanned for each eye, and biometric parameters were measured in hospital. Statistical analyses were performed with the mean values of above parameters at the regions of prelamina, lamina cribrosa, peripapillary sclera, and peripapillary nerve fiber layer, and biometric parameters of age, axial eye length, refractive error, and intraocular pressure.

In qualitative observation, the lamina cribrosa generally shows more hyper signals in AC, LB, and OCA than prelamina. In t-test, AC, LB, and OCA showed significant difference ($p < 0.05$) between prelamina and lamina cribrosa, while conventional OCT did not. In correlation test, axial eye length is positively correlated with LB and AC in lamina cribrosa. And these LB and AC are also negatively correlated with the refractive error. Age was found to be negatively correlated with OCA in lamina cribrosa.

10045-77, Session 5

Dynamic light scattering of vitreous in patients with vitreous floaters compared to macular pucker

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BACKGROUND: Vitreous opacities and posterior vitreous detachment (PVD) disturb vision by degrading contrast sensitivity (AJO 172:7-12, 2016). Increased light scattering is the presumed mechanism. To test this hypothesis, dynamic light scattering (DLS) was performed on excised vitreous of patients with clinically significant floaters, and compared to macular pucker controls.

METHODS: Undiluted, unfixed vitreous was procured during 25-gauge vitrectomy in 14 subjects (age = 59 ± 6.6 years) with clinically significant

vitreous floaters, and 6 controls (age = 66.5 ± 8.7 years; P = 0.10) with macular pucker. Total protein concentration was determined by fluorescent Quant-iT™ protein assay kit (Invitrogen/Molecular Probes, Eugene, OR) with bovine serum albumin (0500 ng/ml) as a standard. Fluorescence (excitation at 470 nm and emission at 570 nm) was measured using a Gemini XPS Dual-Scanning Microplate Spectrofluorometer and data analyzed using SoftMax® Pro software (Molecular Devices, Sunnyvale, CA). DLS (NS300, Malvern Instruments, Westborough, MA) measurements were performed in each specimen after 10-fold dilution in phosphate buffered saline to optimize concentration in each specimen and determine the mean number of particles, the particle size distributions, and the average particle sizes.

RESULTS: Total protein concentration in vitreous specimens trended higher in macular pucker controls (1037 ± 1038 ?g/mL) than eyes with vitreous floaters (353.7 ± 141.1 ?g/mL; P = 0.08). When normalized to total protein concentration, the number of particles in vitreous from floater eyes was more than 2-fold greater than controls (P < 0.04). Particle size distributions were similarly two-fold greater in vitreous from floater subjects as compared to controls (P < 0.05). The average particle size in vitreous from floater eyes was 315.8 ± 194.6 nm, compared to 147.7 ± 129.3 nm in macular pucker controls (P = 0.039).

CONCLUSIONS: Vitreous from eyes with clinically significant floaters contains more particles of larger sizes as compared to controls, likely accounting for the degradation of contrast sensitivity previously found in these patients (Retina 34:1062-8, 2014; IOVS 56:1611–7, 2015; AJO 172:7-12, 2016). DLS could elucidate the underlying molecular abnormalities in patients afflicted with bothersome vitreous floaters and help develop clinical tools to better measure vitreous floaters as well as test the efficacy of various therapies.

10045-27, Session 6

Motion-corrected optical coherence tomography angiography imaging

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Optical coherence tomography angiography (OCT-A) technique can provide depth-resolved images of ocular circulation non-invasively, which is very important not only for ophthalmic diagnosis but also for the study of eye diseases such as glaucoma, diabetic retinopathy and age-related macular degeneration. However, the eye motion corrupted the OCT-A images by creating some motion artifacts which may make the blood vessels discontinuous or distort the shape of them in OCT-A images.

In this study, we developed a 3D motion-corrected OCT-A imaging method for imaging ocular circulation. Firstly, we modified the original Lissajous scan pattern to make it suitable for OCT-A measurement. Secondly, the OCT-A images were acquired with a complex mapping algorithm for optical coherence angiography (cmOCA) using Jones-matrix optical coherence tomography (JM-OCT). Thirdly, the amount of lateral and axial motion shifts of each A-line was calculated. Fourthly, we applied these shifts amount to correct motion artifacts in OCT-A volume data.

To obtain OCT volume data, a 1-?m band JM-OCT with an A-line rate of 100 kHz and 1.4mW probing power is used. The 4.5mm?4.5mm transverse area is scanned by Lissajous scan with 361 repeated cycles, and each repeated cycle contains 725 A-lines, which takes 5.2 seconds for single volume measurement.

By comparing the OCT-A images before and after motion correction, the effectiveness of our motion correction algorithm will be demonstrated.

10045-28, Session 6

Ultra-wide field OCT angiography by using swept source OCT in living human eye

Qinqin Zhang, Chieh-Li Chen, Zhongdi Chu, Ruikang K. Wang, Univ. of Washington (United States)

To investigate the application of ultra-wide field optical microangiography (OMAG) in living human eye. Patients with different macular diseases were recruited, including non-proliferative diabetic retinopathy (NPDR), PDR and venous occlusion, et al. All subjects scanned by a 1060 nm swept source OCT angiography system with A-line speed of 100 kHz was provided by Carl Zeiss Meditec. Inc with tracking capability for motion correction in real time. Ultra-wide field OCTA images can be generated in a single scan within 5 seconds based on the tracking system. OMAG algorithm based on complex differentiation was used to extract the blood flow. Four layers were automatically segmented and provided by the device in the retina. The en-face maximum projection was used to obtain 2-dimensional angiograms of different layers coded with different colors. Wild-field en-face OMAG images of different macular diseases showed a great agreement with FA. Meanwhile, wild-field OMAG gave more distinct vascular network visions that were less affected by hemorrhage and leakage. Compared with the 2D FA images, the vessel dilation and tortuosity was observed based on OMAG angiograms of venous occlusion patients, which is difficult for FA images due to the leakage. In addition, wide-field OMAG angiograms provided the macular ischemia and non-perfusion area in retinal layer as a dark region in far peripheral region, which is more important for DR patients. Ultra-wide field OMAG provides depth-resolved information and detailed vascular images of venous occlusion and DR patients in far peripheral region, providing a better visualization of vascular network compared to FA.

10045-29, Session 6

Long axial imaging range, wide-field swept source optical coherence tomography and optical coherence tomography angiography

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Using a commercial available 200K swept source laser, we demonstrated high resolution wide field angiographic imaging of human retinal. 8mm by 8mm and 10mm by 6mm retina areas were imaged in a single scan within 4 seconds. By montaging four 10 x 6mm scan, 10 x 20mm wide field OCT angiography images were demonstrated.

10045-30, Session 6

High-resolution line-field OCT angiography using digital aberration correction

Laurin Ginner, Abhishek Kumar, Daniel J. Fechtig, Lara M. Wurster, Rainer A. Leitgeb, Medizinische Univ. Wien (Austria)

We present high resolution functional OCT imaging with a line field spectral domain system. We use a split aperture method to determine defocus and higher phase errors of the recorded volume. To use digital wavefront correction techniques a phase correlation over the whole Volume is necessary. Thus a line field system was used, which enables high B-scan rates, in this case up to 2.5 kHz. With such B-scan rate we showed last year that we can apply higher order phase correction to compensate for phase errors. The overall exposure time was 250 micro seconds and the measured Sensitivity 92 dB. In this paper we demonstrate the impact of such digital wavefront correction techniques on functional OCT angiography. We recorded 4 B-scan on one position and calculate the corresponding angiogram, we used refocusing to regain micro vascular details and their right dimensions. This technique can be used in general to enhance not only the image quality, it furthermore enables to correction forf fine motion artifacts during the measurement. The high-resolution angiograms show furthermore suppressed shadowing artifacts, which is important for an accurate quantitative vascular density map.

10045-31, Session 6

Temporal variations in absolute retinal blood velocity, flow and vessel diameter by three beam Doppler optical coherence tomography

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We present the evaluation of short term temporal variations in absolute blood velocity, flow and diameter in all major retinal vessels emerging from the optic nerve head without a heart rate trigger using a three beam Doppler optical coherence tomography method. A semi automatic algorithm was developed to register time points in different cardiac cycles to allow averaging over several cycles in order to increase the accuracy of the measurement. A clear difference was found for the pulsatility in arteries and veins regarding velocity, flow and diameter.

Normalized averages for all arteries and veins were calculated for the three properties. For arteries, both phases of the pulse signal can be identified with a visible shoulder which might correspond to the dicrotic notch. A distinct phase pattern can also be appreciated for veins. The venous velocity peak occurred 0.28 s after the arterial peak, while the full cardiac cycle lasted 0.83 s. The variation in the blood velocity during one cardiac cycle was around 60% for arteries and 20% for veins.

In conclusion three beam D-OCT is capable of temporally resolving the pulse wave in retinal vessels and of evaluating changes in the absolute velocity. Since the quantification of blood flow alterations is a subject of ongoing studies the quantification of temporal variations may help to better understand the pathophysiology of certain eye diseases.

10045-32, Session 6

4D microscope-integrated intraoperative optical coherence tomography angiography

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Optical coherence tomography (OCT) allows for micron scale imaging of the human retina and cornea. Previous research and commercial intraoperative OCT prototypes have been limited to live B-scan imaging because they were based on previous-generation spectral domain OCT systems. Our group has developed and reported on an intraoperative microscope integrated OCT system based on a 100 kHz commercial swept source laser. This system is capable of live 4D imaging, and with a heads up display allows for dynamic intraoperative visualization of retinal structures, tool tissue interaction, and surgical maneuvers. OCT angiography (OCTA) is an emerging OCT technology that allows for imaging of retinal vasculature without the use of potentially harmful contrast agents. This structural information can provide insights into the state and development of a wide range of ophthalmic pathologies. The addition of OCTA into intraoperative OCT could allow for monitoring of changes in retinal vasculature during surgery and imaging of traditionally non-compliant patients. In this work we provide a brief update of intraoperative 4D MIOCT across a range of pathologies, and demonstrate intraoperative OCTA for the first time. To the best of knowledge, this is the first report of intraoperative OCTA, as well as the first OCTA images ever acquired in an infant.

10045-33, Session 6

Imaging of the human choroid with a 1.7 MHz A-scan rate FDML OCT system

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We will demonstrate OCT angiography and Doppler OCT imaging of the choroid in the eyes of healthy volunteers and in AMD cases. We will show that visualization of specific choroidal layers requires selection of appropriate OCTA methods. We will explore the possibility of Doppler OCT imaging to provide information about directionality of blood flow in choroidal vessels. Selected data visualization strategies will be demonstrated. To achieve these goals, we have developed an OCT system utilizing an FDML laser operating at 1.7 MHz sweep rate, at 1060 nm center wavelength, and with ~5 μm axial imaging resolution. The imaging platform was designed to provide narrow field imaging (4 deg). 9 to 18 overlapping volumes were acquired in different locations of the retina to enable generation of wide field mosaics spanning 5 deg temporal to 13 deg nasal, and 5 deg superior to 5 deg inferior from the fovea. Amplitude decorrelation and phase variance OCTA algorithms were implemented for visualization of the vessels. Joint Spectral and Time domain OCT (STdOCT) method was used for Doppler OCT imaging and for OCTA imaging.

10045-34, Session 7

Aberration correction for human retinal imaging using a combination of hardware and computational adaptive optics

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To acquire high-resolution images of the human retina, it is necessary to overcome patient-specific ocular aberrations. Hardware adaptive optics (HAO) has been highly successful in measuring and correcting the aberrated wavefront backscattered from the retina. In particular, adaptive optics combined with optical coherence tomography (OCT) enables three-dimensional, high-resolution retinal imaging. However, even the best HAO systems leave some residual aberrations uncorrected due to their finite spatial and temporal sampling, which ultimately limit retinal image quality. Computational adaptive optics (CAO) is a post-processing method that digitally manipulates the wavefront of the complex OCT data to correct aberrations. With sufficient phase-stability, this method can also provide aberration-corrected images of the human retina. However, as a post-processing method, CAO does not physically modify the optical wavefront, but relies upon the imaging system to collect sufficient photons at the time of data acquisition. This can be quite challenging when imaging highly aberrated eyes with a standard OCT system. We combine both HAO and CAO to address the shortcomings of each method. Using a high-speed OCT system, a wavefront sensor and deformable mirror are used to physically compensate for aberrations and enhance light collection to acquire a three-dimensional, high-SNR dataset. This data is then further optimized using CAO to correct residual aberrations. These results demonstrate the complimentary nature of HAO and CAO to provide high-SNR, high-resolution images optimized for multiple layers of the human retina.

10045-35, Session 7

Fast 3D Strip-Wise Registration of AO-OCT Retinal Volumes with GPU

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Recent development of AO-OCT technology has enabled non-invasive, high resolution imaging of the cellular retina in 3D, which offers the promise of earlier detection of eye diseases. However, high-resolution volumetric imaging generates an enormous amount of data. Moreover, the data is corrupted throughout by random eye motion artifacts due to involuntary eye movements. To correct for these motion artifacts in post processing, we present a novel strip-wise registration algorithm that operates in 3D and execute it at high speed by GPU implementation. The method bases on 3D intensity and phase correlations of individual fast B-scans in target and reference volumes. Each fast B-scan in a target volume is matched to its corresponding B-scan in a reference volume along with XYZ motion displacements between the two, which are then used to align the B-scans and compensate eye motion in both lateral and depth dimensions. To increase speed, registration was implemented using a coarse-to-fine B-scan search that provides accurate prediction of the exact B-scan location without searching the whole reference volume. Our method achieves not only high registration accuracy, robustness, and temporal bandwidth, but also significant speedup compared to CPU implementation. To register a volume of size 512 x 512 x 512, GPU implementation only takes 10.6 s, which is an 18X speedup. Using our 3D registration method, we can even track fast eye motions such as micro-saccades, which was not possible with previous stripe-wise registration methods based on 2D projected images.

10045-36, Session 7

Wide-field human photoreceptor morphological analysis using phase-resolved sensorless adaptive optics swept source OCT

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Adaptive optics optical coherence tomography (AO-OCT) systems capable of 3D high resolution imaging have been applied to posterior eye imaging in order to resolve the fine morphological features in the retina. Human cone photoreceptors have been extensively imaged and studied for the investigation of retinal degeneration resulting in photoreceptor cell death. However, there are still limitations of conventional approaches to AO in the clinic, such as relatively small field-of-view (FOV) and the complexities in system design and operation.

In this research, a recently developed phase-resolved Sensorless AO Swept Source based OCT (SAO-SS-OCT) system which is compact in size and easy to operate is presented. Owing to its lens-based system design, wide-field imaging can be performed up to 6° on the retina. A phase stabilization unit was integrated with the OCT system. With the phase stabilized OCT signal, we constructed retinal micro-vasculature image using a phase variance technique. The retinal vasculature image was used to align and average multiple OCT volumes acquired sequentially. The contrast-enhanced photoreceptor projection image was then extracted from the averaged volume, and analyzed based on its morphological features through a novel photoreceptor structure evaluation algorithm.

The retinas of twelve human research subjects (10 normal and 2 pathological cases) were measured in vivo. Quantitative parameters used for evaluating the cone photoreceptor mosaic such as cell density, cell area, and mosaic regularity are presented and discussed. The SAO-SS-OCT system and the proposed photoreceptor evaluation method has significant potential to reveal early stage retinal diseases associated with retinal degeneration.

10045-37, Session 7

Full-depth imaging of the photoreceptor-RPE-choriocapillaris complex using MHz AO-OCT

Kazuhiro Kurokawa, Zhuolin Liu, Donald T. Miller, Indiana Univ. (United States)

Photoreceptors, retinal pigment epithelium (RPE) and choriocapillaris (CC) compose a complex of neural and support cells that are tightly interconnected to provide the first step of vision. But disturbances in these interconnections can lead to a host of retinopathies, most notably age-related macular degeneration. Histopathological studies have shown that disease onset in the complex begins at the molecular and cellular levels and thus detection and monitoring of changes at this scale provides the greatest promise for improved diagnosis and intervention. Optical coherence tomography equipped with adaptive optics (AO-OCT) has been shown to be a promising noninvasive method to meet this challenge, but a major bottleneck is its failure to image simultaneously cellular details across the full depth of the complex. Here we address this bottleneck with an improved AO-OCT based method that acquires volumes at megahertz A-scan rates in the living human eye and from which simultaneous registered, averaged intensity and angiographic images of the complex are obtained. We demonstrate that morphometric parameters of photoreceptors, RPE cells, and CC capillaries can be extracted from the same volumes. To the best of our knowledge, these are the first images in the living human eye that preserve one-to-one mapping of cellular structure across the full depth of the photoreceptor-RPE-CC complex. Taking advantage of this mapping, cone and RPE cell prevalence directly above CC capillaries was measured at 56% and 52%, respectively, suggesting oxygen and nutrients are uniformly diffused across the complex in the normal, healthy retina.

10045-38, Session 7

Characterizing motility dynamics in human RPE cells

Zhuolin Liu, Kazuhiro Kurokawa, Furu Zhang, Donald T. Miller, Indiana Univ. (United States)

Retinal pigment epithelium (RPE) cells are vital to health of the outer retina, however are often compromised in ageing and ocular diseases that lead to blindness. Early manifestation of RPE disruption occurs at the cellular level, but while in vivo biomarkers at this scale hold considerable promise, RPE cells have proven extremely challenging to image in the living human eye. Recently we addressed this problem by using organelle motility as a novel contrast agent to enhance the RPE cell in conjunction with 3D resolution of adaptive optics-optical coherence tomography (AO-OCT) to section the RPE layer [1]. In this study, we expand on the central novelty of our method – organelle motility – by characterizing the dynamics of the motility in individual RPE cells, important because of its direct link to RPE physiology. To do this, AO-OCT videos of the same retinal patch were acquired at approximately 1 min intervals, time stamped, and registered in 3D with sub-cellular accuracy. Motility was quantified by an exponential decay time constant, the time for motility to decorrelate the speckle field across an RPE cell. In four normal subjects, RPE motility decorrelated speckle in 2.70±0.36 s. This value has two fundamental implications. First, it establishes a preliminary baseline to which motility of diseased RPE cells can be compared. Second, it predicts that the 90-min imaging protocol reported earlier by us for individuating RPE cells can be reduced by 60?, down to just 1.5 min and now clinically viable.

[1] Liu, et al. IOVS, 57(9), OCT533-OCT543, (2016).

10045-39, Session 7

Investigation of retinal microstructure in healthy eyes and dry age-related macular degeneration using a combined AO-OCT-SLO system

Elaine M. Wells-Gray, Stacey S. Choi, The Ohio State Univ. (United States); Matthew Ohr, Colleen M. Cebulla, The Ohio State Univ. Havener Eye Institute (United States); Nathan Doble, The Ohio State Univ. (United States)

Combined adaptive optics (AO) optical coherence tomography (OCT) scanning laser ophthalmoscopy (SLO) imaging allows simultaneous en face and cross sectional views of the retina. We describe improvements to our AO-OCT-SLO system and highlight its clinical utility by presenting results from both three healthy and four dry age-related macular degeneration (AMD) subjects. The AO-OCT-SLO system acquires SLO frames (680 ± 3 nm) and OCT B-scans (860 ± 70 nm) simultaneously at 60 Hz, with the field of view ranging from 0.7°?0.5° to 1.0°?1.5°. The AO system uses a 97 actuator deformable mirror and Shack-Hartmann wavefront sensor, with the OCT used for wavefront correction. For the control group, OCT A-scans were grouped as originating from cones or rods and averaged, and the resulting reflectance profiles were used to identify retinal features. Results for rods and cones were compared, with focus on inner segment (IS) and outer segment (OS) structure and retinal pigment epithelium embedding. In the AMD patients, cone IS and OS lengths were measured over and around drusen for two regions of eccentricity, and those results correlated to drusen height. From the fovea to 2°, the cut-off drusen height that correlated with shortened cone ISL and OSL were 50 μm and 20 μm respectively. For 3°-6° region, the equivalent cut-off drusen heights were 60 μm for ISL and 30 μm for OSL. Results from these studies indicate that the combined AO-OCT-SLO system is capable of high resolution imaging of retinal microstructure in both healthy and diseased eyes.

10045-40, Session 7

Tracking dynamics of photoreceptor disc shedding with adaptive optics optical coherence tomography

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Absorption of light by photoreceptors initiates vision, but also leads to accumulation of toxic photo-oxidative compounds in the photoreceptor outer segment (OS). To prevent this buildup, small packets of OS discs are periodically pruned from the distal end of the OS, a process called disc shedding. Unfortunately dysfunction in any part of the shedding event can lead to photoreceptor and RPE dystrophy, and has been implicated in numerous retinal diseases, including age related macular degeneration and retinitis pigmentosa. While much is known about the complex molecular and signaling pathways that underpin shedding, all of these advancements have occurred in animal models using postmortem eyes. How these translate to the living retina and to humans remain major obstacles. To that end, we have recently discovered the optical signature of cone OS disc shedding in the living human retina, measured noninvasively using optical coherence tomography equipped with adaptive optics in conjunction with post processing methods to track and monitor individual cones in 4D. In this study, we improve on this method in several key areas: increasing image acquisition up to MHz A-scan rates, improving reliability to detect disc shedding events, establishing system precision, and developing cone tracking for use across the entire awake cycle. In a series of three experiments we use this optical signature to measure the temporal and spatial properties of cone shedding dynamics in three normal subjects. To the best of our knowledge this the first observation of photoreceptor disc shedding in the living retina.

10045-41, Session 8

Master/slave based optical coherence tomography for in-vivo, real-time, long axial imaging range of the anterior segment

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In this report we demonstrate that in a coherence revival (CR) based swept source optical coherence tomography (SS-OCT) set-up, real-time cross-sectional long-range images can be produced via the Master Slave (MS) method. The total tolerance of the MS method to nonlinear tuning, to dispersion in the interferometer and to dispersion due to the laser cavity, makes the MS ideally suited to the practice of CR. In addition, enhanced versatility is allowed by the MS method in displaying shorter axial range images than that determined by the digital sampling of the data. This brings an immediate improvement in the speed of displaying cross-sectional images at high rates without the need of extra hardware such as graphics processing units or field programmable gate arrays. The long axial range of the coherence revival regime is proven with images of the anterior segment of healthy human eye.

10045-42, Session 8

Non-contact full-field optical coherence tomography: a novel tool for in vivo imaging of the human cornea

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According to the World Health Organization (WHO), corneal diseases alongside with cataract and retinal diseases are major causes of blindness worldwide. For the 95.5% of corneal blindness cases, prevention or rehabilitation could have been possible without negative consequences for vision, provided that disease is diagnosed early. However, diagnostics at the early stage requires cellular-level resolution, which is not achieved with routinely used Slit-lamp and OCT instruments. Confocal microscopy allows examination of the cornea at a resolution approaching histological detail, however requires contact with a patient's eye. The recently developed full-field OCT technique, in which 2D en face tangential optical slices are directly recorded on a camera, was successfully applied for ex vivo eye imaging. However, in vivo human eye imaging has not been demonstrated yet. Here we present a novel non-contact full-field OCT system, which is capable of

imaging in air and, therefore, shows potential for in vivo cornea imaging in patients. The first cellular-level resolution ex vivo images of cornea, obtained in a completely non-contact way, were demonstrated. We were able to scan through the entire cornea (400 μm) and resolve epithelium, Bowman's layer, stroma and endothelium. FFOCT images of the human cornea in vivo were obtained for the first time. The epithelium structures and stromal keratocyte cells were distinguishable. Both ex vivo and in vivo images were acquired with a large (1.26 mm x 1.26 mm) field of view. Cellular details in obtained images make this device a promising candidate for realization of high-resolution in vivo cornea imaging.

10045-43, Session 8

Truly simultaneous SS-OCT of the anterior and posterior human eye with full anterior chamber and 50° retinal field of views

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Optical coherence tomography (OCT) has revolutionized clinical observation of the eye and is an indispensable part of the modern ophthalmic practice. Unlike many other ophthalmic imaging techniques, OCT provides three-dimensional information about the imaged eye. However, conventional clinical OCT systems image only the anterior or the posterior eye during a single acquisition. Newer OCT systems have begun to image both during the same acquisition but with compromises such as limited field of view in the posterior eye or requiring rapid switching between the anterior and posterior eye during the scan. We describe here the development and demonstration of an OCT system with truly simultaneous imaging of both the anterior and posterior eye capable of imaging the full anterior chamber width and 50° on the retina (macula, optic nerve, and arcades).

The whole eye OCT system was developed using custom optics and optomechanics. Polarization was utilized to separate the imaging channels. We utilized a 200kHz swept-source laser (Axsun Technologies) centered at 1040±50nm of bandwidth. The clock signal generated by the laser was interpolated 4x to generate 5504 samples per laser sweep. With the whole eye OCT system, we simultaneously acquired anterior and posterior segments with repeated B-scans as well as three-dimensional volumes from seven healthy volunteers (other than refractive error). On three of these volunteers, whole eye OCT and partial coherence interferometry (LenStar PCI, Haag-Streit) were used to measure axial eye length. We measured a mean repeatability of ±47μm with whole eye OCT and a mean difference from PCI of -68μm.

10045-44, Session 8

Dual wavelength scanning light ophthalmoscope using digital micromirror device

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Scanning Light Ophthalmoscope (SLO) has grown into a vital diagnostic tool in ophthalmology by providing visualisation of retinal structures. Multispectral imaging is an emerging modality for ophthalmic applications, but the majority of such imaging systems are either based on a fundus camera offering with low resolution or uses expensive components such as supercontinuum source and detector arrays. Here, we present a compact dual wavelength SLO for in-vivo retinal imaging using a Digital Micromirror Device (DMD).

The current version of our system uses the DMD to create concentric circles to scan the retina. The reflected light is collected using a CMOS camera, and the confocal image is produced in post-processing. We obtained confocal images of a healthy subject using 810 nm illumination with an imaging speed of 8Hz and the time averaged optical power on the cornea was 150?W. We demonstrated multispectral imaging (810nm and 650nm) on a model eye consisting of a retina made of two layers of TiO2 (attenuation coefficient of 10mm-1, thickness of 170 microns). A black ink having a relatively strong absorption at 650 nm was used to draw lines between these layers to highlight intensity variation due to differential absorption. The concentric ring pattern was projected at 150 Hz (fill factor of 1/25 corresponding to imaging speed of 3 Hz for two wavelengths)

The modular design of the system allows imaging using different wavelengths at a low cost. By combining the advantages offered by the DMD technology and multispectral imaging, we aim to make the imaging system suitable for in-vivo imaging for diagnosis and screening of eye diseases.

10045-45, Session 8

Optical photon reassignment super-resolved scanning laser ophthalmoscopy

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Conventional scanning laser ophthalmoscopy (SLO) utilizes a finite collection pinhole at a retinal conjugate plane to strongly reject out-of-focus light while primarily transmitting the in-focus, retinal backscattered signal. However, to improve lateral resolution, a sub-Airy disk collection pinhole is necessary, which drastically reduces the signal-to-noise ratio (SNR) of the system and is thus not commonly employed. Recently, an all-optical, super-resolution microscopy technique known as optical photon reassignment (OPRA) microscopy (also known as re-scan confocal microscopy) has been developed to bypass this fundamental tradeoff between resolution and SNR in confocal microscopy. We present a methodology and system design for obtaining super resolution in retinal imaging by combining the concepts of SLO and OPRA microscopy. The resolution improvement of the system was quantified using a 1951 USAF target at a telecentric intermediate image plane. Retinal images from human volunteers were acquired with this system both with and without using the OPRA technique to demonstrate the resolution improvement when imaging parafoveal cone photoreceptors. Finally, we quantified the resolution improvement in the retina by analyzing the radially averaged power spectrum of the retinal images.

10045-46, Session 8

Modular multimodal swept-source spectrally encoded scanning laser ophthalmoscopy and optical coherence tomography scan-head for surgical microscope-integrated and slit-lamp imaging

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Scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT) enable noninvasive in vivo diagnostic imaging and provide complementary en face and depth-resolved visualization of ophthalmic structures, respectively. We previously demonstrated concurrent multimodal swept-source spectrally encoded scanning laser ophthalmoscopy and OCT (SS-SESLO-OCT) at 1060 nm using a swept-source and double clad fiber coupler. Here, we present system enhancements and novel designs for a modular SS-SESLO-OCT scan-head that can be coupled to ophthalmic surgical microscope-integrated and slit-lamp imaging optics. Multimodal

SS-SESLO-OCT was demonstrated using a custom-built swept-source OCT engine with a 200 kHz 1060 nm source that was optically buffered for concurrent SESLO and OCT imaging at 100% duty cycle and 400 kHz sweep-rate. A shared optical relay and fast-axis galvanometer ensured inherent co-registration between SESLO and OCT field-of-views and concurrent acquisition of an en face SESLO image with each OCT cross-section. SESLO and OCT frames were acquired at 200 fps with 2560 x 2000 pix. (spectral x lateral). We show in vivo human ophthalmic imaging data using surgical microscope-integrated and slit-lamp imaging relays to demonstrate the utility of our SS-SESLO-OCT design. Our self-contained modular scan-head can be used for either intraoperative guidance or clinical diagnostics and reduces the complexity, cost, and maintenance required for clinical translation of these technologies. We believe concurrent multimodal SS-SESLO-OCT may benefit 1) intraoperative imaging by allowing for real-time surgical feedback, instrument tracking, and overlays of computationally extracted image-based surrogate biomarkers of disease, and 2) slit-lamp imaging by enabling aiming, image registration, and multi-field mosaicking.

10045-47, Session 8

1050 nm diagnostic imaging of pediatric retinoblastoma patients with a novel handheld optical coherence tomography system

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We discuss the clinical study with a novel 1050 nm optical coherence tomography system specifically implemented and validated for imaging of retinoblastoma tumors in pediatric patients. The treatment options for this malignant tumor of the retina focus on reduction of tumor recurrence risks, and vision preservation. The choice and success of treatment strongly depend on accuracy of clinical assessment and skills of the clinician. The children with risk for retinoblastoma and the treated patients are monitored periodically by retinal imaging under anesthesia due to limitations of the existing real-time diagnostic tools: funduscopy, ultrasound, and MRI.

The three-dimensional visualization of tissue morphology and microvasculature at detailed axial and lateral resolution of OCT imaging enhances sensitivity of real-time diagnostics, helps distinguish vital tumor masses from similarly looking non-malignant disorders, and improves the treatment strategy. Our design accommodates for the range of optical parameters of children eyes. The handheld imaging module is implemented in non-contact mode for patients in supine position under general anesthesia. Mechanically tunable lens provides focusing, where resolution is 6 μm axially, and varies with between 10-18μm laterally.

The findings of the ongoing clinical study will be discussed, specifically the images of early, active, and treated retinoblastoma tumors, differentially diagnosed disorders, and follow-up observations. We will demonstrate that ambiguous diagnostic questions are better addressed with three-dimensional visualization by non-invasive OCT imaging.

10045-48, Session 9

Combined laser-ray tracing and OCT system for biometry of the crystalline lens

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Age-related changes in the crystalline lens shape and refractive index gradient produce changes in dioptric power and high-order aberrations that influence the optics of the whole eye and contribute to a decrease in overall visual quality. Despite their key role, the changes in lens shape and refractive index gradient with age and accommodation and their effects on high-order aberrations are still not well understood. The goal of this project was to develop a combined laser ray tracing (LRT) and optical coherence tomography (OCT) system to measure high-order aberrations, shape and refractive index gradient in non-human primate and human lenses. A miniature motorized lens stretching system was built to enable imaging and aberrometry of the lens during simulated accommodation. A positioning system was also built to enable on- and off-axis OCT imaging and aberrometry for characterization of the peripheral defocus of the lens. We demonstrated the capability of the LRT-OCT system to produce OCT images and aberration measurements of crystalline lens with age and accommodation in vitro. In future work, the information acquired with the LRT-OCT system will be used to develop an accurate age-dependent lens model to predict the role of the lens in the development of refractive error and aberrations of the whole eye.

10045-49, Session 9

Synchronous imaging of the pulse response of the ciliary muscle and lens with SD-OCT

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Purpose: To determine the dynamic interaction between ciliary muscle and lens during accommodation and disaccommodation through synchronous

imaging of ciliary muscle and lens response to pulse stimulus

Methods: The ciliary muscle and lens were imaged simultaneously in a 33 year old subject responding to a 4D pulse stimulus (accommodative stimulus at 1.7 s, disaccommodative stimulus at 7.7 s) using an existing imaging system (Ruggeri et al, 2016) consisting of an Anterior Segment Optical Coherence Tomography system, Ciliary Muscle Optical Coherence Tomography system, and custom-built accommodation module. OCT images were recorded at an effective frame rate of 13.0 frames per second for a total scan time of 11.5 s.

An automated segmentation algorithm was applied to images of the anterior segment to detect the boundaries of the cornea and lens, from which lens thickness was extracted. Segmentation of the ciliary muscle was performed manually and then corrected for distortion due to refraction of the beam to obtain measurements of thicknesses at the apex and fixed distances from the scleral spur.

Results: The dynamic biometric response to a pulse stimulus at 4D was determined for both the ciliary muscle and lens, suggesting the ciliary muscle and lens interact differently in accommodation and disaccommodation.

Conclusions: The study introduces new data and analyses of the ciliary muscle and lens interaction during a complete accommodative response from the relaxed to the accommodated state and back, providing insight into the interplay between individual elements in the accommodative system and how their relationships may change with age.

10045-50, Session 9

Three-dimensional assessment of crystalline lens opacities in cataract patients using long-depth-range SS-OCT

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Cataract is one of the leading causes of blindness worldwide since it affects the visual quality due to opacifications in the crystalline lens. We demonstrate three-dimensional (3-D) visualization of crystalline lens opacities in vivo in patients with different types and grades of cataract. We developed a prototype long-depth-range Swept-Source OCT instrument operating at the speed of 50 kA-scans/second and at the central wavelength of 1 ?m to perform high-resolution imaging of the whole anterior segment of the eye. Volumetric data sets of 19 cataractous eyes were acquired and processed to obtain contrast-enhanced high-resolution images of lenticular opacifications. The results showed lens micro-scale features related to possible cataract development such as cortical spokes (wedge-shaped opacities), waterclefts or enhanced scattering in lens nucleus etc. The results also demonstrate the ability of the OCT imaging to characterize the opacities quantitatively. To conclude, 3-D long-depth-range SS-OCT enables volumetric visualization of in vivo microstructural changes in the crystalline lens related to opacification. The instrument might be a useful tool in the high-resolution evaluation and surgical management of crystalline lens opacities in cataract patients.

10045-51, Session 9

Predicting post-cataract surgery refractive outcome error using extended-depth OCT, corneal biometry, and a paraxial optical model of the pseudophakic eye

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As a step towards understanding the sources of errors in intraocular lens (IOL) power calculations, the goal of the present study was to determine if the refractive state of the pseudophakic eye can be predicted using a patient-customized paraxial model of the eye based on ocular biometry alone, without the need for correction factors.

In an IRB-approved study, 11 eyes of 7 subjects who underwent cataract surgery and IOL implantation underwent subjective refraction, corneal topography and extended-depth SD-OCT imaging with a system developed in-house. The measurements were entered in a patient-customized eye model consisting of two thin lenses representing the cornea and IOL. The position of the retinal conjugate was calculated to determine the predicted postoperative refractive outcome. The difference between predicted refraction and the measured postoperative spherical equivalent was calculated for each subject.

The prediction error was within +/-0.5 D in 10 out of 11 eyes and within +/-0.25 D in 7 out of 11 eyes.

The study shows that the refractive state of the pseudophakic eye can be accurately predicted from optical biometry using a simple paraxial model of the eye, without the use of correction factors.

10045-52, Session 9

Correction of hyperopia by intrastromal cutting and biocompatible filler injection

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For ametropic eyes, LASIK is a common surgical procedure to correct the refractive error. However, the correction of hyperopia is more difficult than that of myopia because the increase of the central corneal curvature by excimer ablation is only possible by intrastromal tissue removal within a ring-like zone in the corneal periphery. For high hyperopia, the ring-shaped indentation leads to problems with the stability and reproducibility of the correction due to epithelial regrowth.

Recently, it was shown that the correction of hyperopia can be achieved by implanting intracorneal inlays into a laser-dissected intrastromal pocket. In this paper we demonstrate the feasibility of a new approach in which a transparent, and biocompatible liquid filler material is injected into a laser-dissected corneal pocket, and the refractive change is monitored via OCT. This technique allows for a precise and adjustable change of the corneal curvature.

Precise cutting of the intrastromal pocket was achieved by focusing UV laser picosecond pulses from a microchip laser system into the cornea. After laser dissection, the transparent filler material was injected into the pocket. The increase of the refractive power by filler injection was evaluated by taking OCT images from the cornea. With this novel technique, it is possible to

precisely correct hyperopia of up to 10 diopters. An astigmatism correction is also possible by using ellipsoidal intrastromal pockets.

10045-53, Session 9

Low-cost, smartphone based frequency doubling technology visual field testing using virtual reality

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Glaucoma is the leading cause of irreversible blindness worldwide. Due to its wide prevalence, effective screening tools are necessary. The purpose of this project is to design and evaluate a system that enables portable, cost effective, smartphone based visual field screening based on frequency doubling technology. The system is comprised of an Android smartphone to display frequency doubling stimuli and handle processing, a Bluetooth remote for user input, and a virtual reality headset to simulate the exam. The LG Nexus 5 smartphone and BoboVR Z3 virtual reality headset were used for their screen size and lens configuration, respectively. The system is capable of running the C-20, N-30, 24-2, and 30-2 testing patterns. Unlike the existing system, the smartphone FDT tests both eyes concurrently by showing the same background to both eyes but only displaying the stimulus to one eye at a time. Both the Humphrey Zeiss FDT and the smartphone FDT were tested on five subjects without a history of ocular disease with the C-20 testing pattern. The smartphone FDT successfully produced frequency doubling stimuli at the correct spatial and temporal frequency. Subjects could not tell which eye was being tested. All five subjects preferred the smartphone FDT to the Humphrey Zeiss FDT due to comfort and ease of use. The smartphone FDT is a low-cost, portable visual field screening device that can be used as a screening tool for glaucoma.

10045-54, Session 9

Chromatic multifocal pupillometry for objective perimetry in patients with Best's Vitelliform Macular Dystrophy

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Purpose: Objective pupilloperimetry in healthy subjects and patients with Best's Vitelliform Macular Dystrophy using a chromatic multifocal pupillometer (CMP).

Methods: A CMP (Accutome Inc) was used to record pupillary responses (PR) of 17 healthy subjects and 5 Best's patients. Red and blue light stimuli were presented at 76 locations of a 16.2 degree VF. The PR of patients were compared with their findings on Humphrey's 24-2 perimetry, Optical Coherence Tomography (OCT) and with the PR of healthy subjects. Percentage of Pupil Constriction (PPC), maximal constriction velocity (MCV) and the latency of MCV (LMCV) were determined.

Results: In response to red light, Best's patients demonstrated reduced PPC and slower MCV compared with healthy subjects in nearly all test point locations. Severity of defects in PPC in responses to red light correlated with reduced thickness of photoreceptor layer as determined by OCT in the central, superior, nasal and temporal areas of the central retina (Pearson's r= -0.93, -0.91, -0.92, -0.85, respectively). By contrast, in response to blue light stimuli, the PPC and MCV of patients were lower than normal only in several central points. Surprisingly, the latency of MCV was shorter in patients compared with healthy subjects in response to red and blue stimuli.

Conclusions: This study demonstrates the potential feasibility of using pupillometer-based chromatic perimetry for objectively assessing VF defects in patients with Best's macular dystrophy. Our findings also suggest that chromatic pupilloperimetry may differentiate between PR mediated by cones or rods, and can specifically detect defects in macular cones.

10045-55, Session PSun

Combining retinal nerve fiber layer thickness with individual retinal blood vessel locations allows modeling of central vision loss in glaucoma

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Purpose: To assess whether modeling of central vision loss (CVL) due to glaucoma by optical coherence tomography (OCT) retinal nerve fiber (RNF) layer thickness (RNFLT) can be improved by including the location of the major inferior temporal retinal artery (ITA), a known correlate of individual RNF geometry.

Methods: Pattern deviations of the two locations of the Humphrey 24-2 visual field (VF) known to be specifically vulnerable to glaucomatous CVL and OCT RNFLT on the corresponding circumpapillary sector around the optic nerve head within the radius of 1.73mm were retrospectively selected from 428 eyes of 428 patients of a large clinical glaucoma service. ITA was marked on the 1.73mm circle by a trained observer. Linear regression models were fitted with CVL as dependent variable and VF mean deviation (MD) plus either of (1) RNFLT, (2) ITA, and (3) their combination, respectively, as regressors. To assess CVL over all levels of glaucoma severity, the three models were compared to a null model containing only MD. A Baysian model comparison was performed with the Bayes Factor (BF) as measure of strength of evidence (BF$<$3: no evidence, 3-20: positive evidence, $>$20: strong evidence over null model).

Results: Neither RNFLT (BF=0.9) nor ITA (BF=1.4) alone provided positive evidence over the null model, but their combination resulted in a model with strong evidence (BF=21.4).

Conclusion: While the established circumpapillary RNFLT sector, based on population statistics, could not satisfactorily model CVL, the inclusion of a retinal parameter related to individual eye anatomy yielded a strong structure-function model.

10045-56, Session PSun

Retinal changes associated with blood glucose levels in diabetic patients assessed with OCT

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Diabetes is the leading cause of visual impairment for working-aged adults in the US. We used optical coherence tomography (OCT) to image the eyes of diabetic patients who did not have ready access to eye care. We used custom software to segment the OCT images automatically to determine individual retinal layer thicknesses. The segmentation was verified by a grader, and manual corrections were performed when there

were mistakes. Manual segmentation produces a smoother boundary than the automatic segmentation, so a final automatic "fine tune" was then implemented, so there would be consistency between the manual and automatic segmentation. We then used the results from the segmentation to determine the foveal center automatically by searching for the lowest contiguous region of the inner limiting membrane boundary. Three sample regions were selected for each subject. The first was at the fovea, where total retinal thickness was computed. The second and third regions were 5 deg nasal and 5 deg temporal to the fovea, and in these places individual layers and total retinal thickness were computed. We examined these results, controlling for age, gender, and HbA1c levels. On average, higher HbA1c levels were associated with thicker nerve fiber layer, both nasally and temporally (p=0.001, and p=0.019, respectively). We used a two-way ANOVA to verify that age and gender had no underlying effect on HbA1c levels.

10045-57, Session PSun

Is the resistance to irradiance test on sunglasses standards effective?

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Ocular safety for sunglasses users is mandatory. Aging tests of sunglasses are important to assure safeness lasts during sunglasses life time. The present literature establishes safe limits on the exposure of the eyes to ultraviolet radiation (UVR). Ultraviolet radiation upon the eyes is related to many ocular pathologies. Regarding UV protection for ocular media, the resistance-to-irradiance test of many national standards requires irradiating the lenses for 50 continuous hours with a 450 W solar simulator, providing a correspondingly evaluation of the exposure to the Sun. The main concern of this test is to establish a reliable correspondence between the solar irradiation and the simulator lamp considering that solar irradiation depends on geographical coordinates, time of year and atmospherical conditions. Our calculation indicates that this stress test is ineffective in that present form. Suggestions for the revision of the parameters of these tests are offered to establish safe limits appropriate to the UV irradiance.

10045-58, Session PSun

Accurate method for luminous transmittance and signal detection quotients measurements in sunglasses lenses

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The international standard ISO 12312-1 proposes transmittance tests that quantify how dark sunglasses lenses are and whether or not they are suitable for driving. To perform these tests a spectrometer and a skilled technician are required. In this study, we present and analyze an accurate alternative method for performing these measurements using simple components. Using three LEDs and a four-channel sensor we generated weighting functions similar to the standard ones for luminous and traffic light transmittances. From 89 sunglasses lens spectroscopy data, we calculated luminous transmittance and signal detection quotients using our obtained weighting functions and the standard ones. Mean-difference Tukey plots were used to compare the results. All tested sunglasses lenses were classified in the right category and correctly as suitable or not for driving. The greatest absolute errors for luminous transmittance and red, yellow, green and blue signal detection quotients were 0.15%, 0.17, 0.06, 0.04 and 0.18, respectively. This method will be used in a device capable to perform transmittance tests (visible, traffic lights and ultraviolet (UV)) according to the standard. It is important to measure rightly luminous transmittance and relative visual attenuation quotients to report correctly whether or not sunglasses are suitable for driving. Moreover, standard UV requirements depend on luminous transmittance.

10045-59, Session PSun

Sunglasses lenses' transmittance analysis after short-term solar exposure: preliminary evaluation

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Most of sunglasses lenses are manufactured using polymeric materials such as polycarbonate and these lenses receive special coatings and additives for providing efficient UV radiation blocking. According to international standards (ISO 12312-1/2013), sunglasses need to assure adequate UV protection depending on how dark the lenses are and also pass through tests, such as the resistance to radiation test. Although lenses may pass this test, meaning adequate optical properties after a simulated exposition, their behaviour in real conditions, i.e., under long-term solar exposition, was not investigated in literature and evidences show that real use conditions may have different effects than the simulation test. Our purpose is to compare the effects of solar exposition in sunglasses optical properties and compare it to effects caused when using a solar simulator for the same time length. A set of 32 lenses was exposed for three months in an automated panel, which turn the lenses towards the sun and protect them from undesirable weather conditions and in a solar simulator by the equivalent time for three months. Visible and UV transmittance measurements were taken by using a spectrophotometer before, during and after the exposition time for both exposition methods and compared. Results suggest that non-negligible changes for both transmittances occur after exposition and at different rates for the two methods and this information may be used to adapt the standard test and make it more suitable to represent real conditions at which sunglasses are subjected to and need to keep their optical characteristics and protection.

10045-60, Session PSun

High-refractive index polyacrylates based on quinolinone-structures for intraocular lenses

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Intraocular lenses (IOL) have experienced an expanding application over the last decades. Not only they can be used to cure cataract caused blindness, but they are also appointed to ease visual impairments (e.g. -18 – 10 dioptre or astigmatism).[1] These phake IOL require materials with very high refractive indices due to the limited space at the implanting position in the eye of the patient. This enables less invasive operations and such with smaller incisions.[2]

Quinolinone derivates, like carbostyril, are currently known from drug design and as a main structural component of several antibiotics.[3] Although they show high refractive indices and good dispersions they have not yet been used in materials for ophthalmic applications. We synthesized and characterized novel high refractive index polymers containing quinolinones as the main refractive unit of the structure.[4] We showed that it was possible to build quinolinone polymers with high refractive indices up to 1.685 at 589 nm. Using this material it would theoretically be possible to reduce the lens thickness of an IOL to under 40 percent compared to a commercial hydrogel lens with a refractive index of 1.470. We also used the synthesized quinolinone acrylates to create hydrophobic copolymers with improved physical properties and high transmission in the visible spectral range. Besides the good lightfastness these copolymers also showed very low tendencies of glistening.

In conclusion quinolinones show attractive performances for the usage as a component in acrylic copolymers. If the requirements for IOL keep rising in the coming years these monomers could be used to boost the refractive index of ophthalmic polymer compositions.

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10045-61, Session PSun

A hyperspectral imaging system for the evaluation of the human iris spectral reflectance

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Age related macular degeneration (AMD), ocular melanoma and cataracts are the most important leading causes of visual impairment and blindness in the world. Previous studies showed that fundus pigmentation is a key factor for the occurrence of these pathologies. Moreover, it is known that a strong correspondence between fundus and iris pigmentation exists: therefore, the characterization of the pigments distribution across all the iridal layers is crucial to better understand the occurrence of eye pathologies and for their early detection.

Iris color is currently assessed in ophthalmology by using subjective and qualitative methods. Having a quantitative measure of the iris spectral reflectance would allow not only a better classification of the spectral signatures, but also to understand and quantify the distribution of melanin, hemoglobin and oxygen in the iridal layers. In addition, the different anatomical structures and textures of the iris are highlighted at different wavelengths, depending on the depth of penetration of each light component into the iris tissues. We propose a simple optical setup, which exploits a monochrome camera integrated into a standard ophthalmic microscope to perform iris hyperspectral imaging. A monochromator is used as the light source: its spectral range and resolution are 450-950nm and 8-15nm, respectively. Reflectance spectra can be calculated both from discrete areas of the iris and as the average of the whole iris surface. Preliminary results suggest that hyperspectral imaging of the iris can provide much more detailed information with respect to conventional qualitative colorimetric methods.

10045-62, Session PSun

Solar exposure of sunglasses: aging test display

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In previous studies conducted in our lab, we have been investigating the aging effects on sunglasses. Some preliminary results have been indicating changes on the UV protection on the lenses. Therefore, besides irradiating the samples with a proper sun simulator, we have also been concerned on exposing the sunglasses to natural sun for further investigation and comparisons. Thus, this project aims expose the lenses for 24 months using a prototype, which consists of a panel (display) with cover, housing 100 lenses arranged in the vertical position to the ground, fixed on a rotating axis, which will be irradiated by the sun from sunrise until sunset. The lid opens and turns the panel towards the sun automatically, so that the lens will always be facing the sun. Humidity, dust, time and UV index sensors, as well as a video camera are part of the system. The exposure time and UV index will be recorded and automatic opening or closing the lid may also be controlled by a PC using online software. The tests are being conducted for three months already and previously to irradiation, spectroscopy was performed and then will be repeated every 30 days of exposure. After three months spectral changes have been observed. These non-negligible changes obtained in short exposure time are promising data, justifying an investigation with longer exposure time.

10045-63, Session PSun

Probing superstructure of chicken corneal stroma by Fourier transform second harmonic generation microscopy

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In this work, we used the combination of the second harmonic generation (SHG) microcopy and Fourier-transform analysis to investigate the three-dimensional superstructure of adult chicken corneas. Our results show that the stroma lamella in anterior stroma rotate in a counterclockwise fahsion and that , the posterior stroma maintains a non-rotating pattern with increasing depth. In addition, the thickness of the anterior stroma remains almost constant throughout the temporal-nasal direction. Through quantitative analysis, the natural transition of the anterior and posterior stroma is also determined. These findings enhance our understanding of the collagen-rich tissue in the chicken cornea model. Moreover, the Fourier-transform-based modality, in combination with SHG microscopy, serves as a promising tool to determine collagen alignment in embryonic development, tissue engineering and corneal diseases.

10045-64, Session PSun

Ophthalmic scatterometry

Noam Sapiens, Consultant (United States); Bat-Chen Cohen, Tel-Aviv Univ. (Israel); Ygal Rotenstreich, The Chaim Sheba Medical Ctr., Tel Hashomer (Israel)

Most medical pathologies cause optical properties variations of the affected tissue. Most of current triage methods are based on imaging (e.g. ophthalmoscope, fundus camera, slit lamp, OCT), namely creation of a visual representation of the affected tissue. A scatterometer measures optical properties without creating an image. Nevertheless, a scatterometer measures a distribution of optical properties that enables deduction of a myriad of parameters otherwise undetectable.

Our system used one of three lasers of different wavelengths (532nm, 785nm and 915nm). The beam was expanded to create a parallel beam with uniform spatial distribution in the plane of the eye. The reflected light from the eye is then directed onto both a refractometer and our scatterometric measurement system. The refractometer is used to account for eye aberrations (mainly defocus) in the result analysis. The scatterometer is comprised of an off-axis parabolic mirror and an array detector in its focus. The pattern formed on the array is in a Fourier conjugate plane to the pupil of the eye, therefore it represents the angular distribution of the light emanating from the eye.

The experimental procedure included calibration of the system to allow for absolute power measurement off the array detector as well as for measurement of targets with well-known optical characteristics.

Results include the scatterometric characteristics of different targets at different wavelengths. Compensation of aberrations using a simple convolution algorithm yield results that are readily predicted by theoretical models. Furthermore, distinct signature of different targets is evident.

10045-66, Session PSun

The relationship between 3D morphology of optic disc and spatial patterns of visual field loss in glaucoma

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Purpose: Optic disc tilt defined over 3D optic disc morphology has been shown to be associated with the location of initial glaucomatous damages. In this work, we study the impact of optic cup depth (OCD) on spatial patterns of visual field loss in glaucoma.

Methods: Pairs of reliable Cirrus OCT scans around optic disc and Humphrey visual fields of glaucoma patients without visually significant cataract and age-related macular degeneration were selected. The most recent visit of a randomly selected eye of each patient was chosen. The OCD was automatically calculated on the superior-inferior cross sectional image passing through the optic disc center. The correlations between the mean pattern deviation (PD) of each sector in glaucoma hemifield test (GHT) and OCD were evaluated for all severities glaucoma and mild glaucoma (mean deviation ≥ -5 dB), respectively.

Results: 424 eyes of 424 patients passed the data reliability criteria with 346 mild glaucoma patients. For all severities glaucoma, there was no significant correlation between the mean sector PD and OCD. For mild glaucoma, OCD was uniquely correlated to the mean PD of the inferior pericentral sector ($r=-0.18$, $p=0.01$) in GHT, which was independent of mean deviation and retinal nerve fiber layer thickness ($p<0.001$ for both).

Conclusion: OCD was uniquely correlated to the vision loss of the inferior pericentral sector in GHT for mild glaucoma. Future advancement of OCT imaging techniques may provide better clinical diagnosis for early glaucoma by focusing on 3D morphological variation of the optic disc.

10045-67, Session PSun

Wide-field fundus imaging with trans-palpebral illumination

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In conventional fundus imaging devices, light delivery into the back of the eye is performed by transpupillary illumination. For this method, light source is directed into the posterior segment of the eye through cornea and passes the pupillary area. As a result of sharing the pupillary area by illumination beam and observation path, pupil dilation is typically necessary for fundus examination and the field of view is limited. An alternative approach is delivering light from the sclera. It is possible to image wider retinal area with transcleral-illumination. Mandatory requirement of physical contact between illumination system and sclera is a drawback of this method. We report a technique, by delivering the light through the upper eyelid, i.e., palpebra. In our experimental setup, we used a 1.5 mm diameter fiber illuminator with a warm white light emission diode (LED). To illuminate inside the eye, the fiber illuminator was placed at a location on the upper eyelid corresponding to pars plana region. A custom made adaptor, containing a 90 diopter ophthalmic lens and a 25 diopter relay lens was attached to the digital camera. The ophthalmic lens collected light coming from the eye and formed an aerial image between the ophthalmic and relay lenses. The aerial image was captured by the camera through the relay lens. Adequate illumination level was obtained to capture wide angle fundus images within ocular safety limits defined by the ISO 15007-2: 2007 standard. This novel trans-palpebral illumination approach enables wide-angle fundus photography without eyeball contact and pupil dilation.

10045-68, Session PSun

Eye tracker in distance estimation

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This paper demonstrates an interesting eye tracker that can accurately estimate the distance of the object where the subjects are gazing at. The device is intended for future autofocus eyeglass that is based on adaptive lens. Conventional eye tracker techniques are usually limited to detection of 2D information. With the similar principle, two near infrared LEDs and two mini-cameras, one for each eye, are mounted on the frame. Images taken by the cameras are processed to find the center of the pupil and the center of the glint. Then the distance between the center of the glint and the center of the pupil for each eye is used to calibrate the gazing angles. Finally the distance of the gazing point is calculated. In the experiments, we have got excellent results in estimation of the gazing distance of different vision tasks. Our estimation results were 36.97cm, 79.02cm and 431.3cm when targets were placed at the distances of 30cm, 70cm, and 400cm, respectively. The device has been demonstrated effectively for a wide field of view (FoV). It's also possible to test the maximum FoV by moving the testing targets. The accuracy and reliability of this technique have been verified experimentally. This technique has great potential in autofocus eyeglass for adaptive vision correction.

10045-69, Session PSun

Chromatic multifocal pupillometer for objective early diagnosis of mild cognitive impairment

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Purpose: To evaluate the use of chromatic multifocal pupillometer (CMP) for objective diagnosis of neurodegeneration in the brain.

Methods: A CMP (Accutome, Inc.) was used to record pupillary responses to red and blue light presented at 76 different locations of a 16.2-degree visual field. Maximal percentage of pupil constriction (PPC), maximal constriction velocity (MCV) and the latency of MCV (LMCV) were determined. Fifteen cognitively normal subjects (ages 60-74) with no detected ophthalmic pathology were included. The CMP results were associated with cognitive (Montreal Cognitive Assessment, MoCA) testing.

Results: Subjects with low MoCA (<26) presented weaker and sluggish pupil responses in peripheral and central locations of the visual field. Low MoCA was associated with 2-3 fold lower PPC in response to red light compared to normal MoCA (≥26), mostly in the central, nasal and central locations. Subjects with MoCA <26 compared to MoCA ≥26 showed reduced PPC in response to blue light in all regions except the inferior (nasal 5.8% vs 17.1% $p=0.01$; temporal 4.4% vs 16.5% $p=0.01$; superior 5.3% vs 16.5% $p=0.02$).

Conclusions: This study demonstrated the feasibility of using the CMP device for identification of functional focal defects associated with cognitive impairment. Changes in pupil response to focal chromatic light stimuli may present early biomarkers for incipient cognitive decline that can be measured using non-invasive low cost techniques. These biomarkers may serve and Alzheimer Disease prevention clinical trials, both for identification

of asymptomatic high risk patients, and as sensitive outcome measure of therapeutic effects.

10045-70, Session PSun

Application of optical coherence tomography angiography for oxygen-induced retinopathy in the rat

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Optical Coherence Tomography Angiography (OCTA) has been widely used in ophthalmology thanks to its capability to observe vascular disorders for both clinical and pre-clinical applications. Due to their ease of maintenance and availability, the rodent models are actively used for various retinal disease studies in pre-clinical levels. This work aims for the application of OCTA in study of oxygen-induced retinopathy (OIR) which induces ischemic vascular disease in the retina.

OIR is one of the most popular models that generates retinal angiogenesis and neovascularization in rodent model. By exposing rodent pups to high oxygen concentration environment while they are still in developmental process, normal vascular formation is obliterated leaving capillary free region especially near the artery. After returning to room condition, those insufficiently perfused areas become hypoxic and trigger angiogenesis and neovascularization. In this study, two groups (control and OIR) of Sprague Dawley rat were prepared. The mother and pups in the OIR group were incubated in 80% oxygen (hyperoxia) chamber right after birth and returned to room at 12 days-old (P12). Meanwhile the control group was remained in the room condition.

For OCT imaging, 1050 nm SS-OCT system was developed for retinal imaging in the rat. The neovascular tufts which is a hallmark of ischemic retina vascular disorder were clearly identified in both OCT intensity and angiography image. The shadow of hyaloid vessel on the OCT angiography and insufficient imaging field of view remain as further discussion.

10045-71, Session PSun

Imaging microstructures of rat cornea using Micro Optical Coherence Tomography (μOCT)

Si Chen M.D., Linbo Liu, Xinyu Liu Sr., Xianghong Wang, Xiaojun Yu, Nanshuo Wang, Nanyang Technological Univ. (Singapore)

Corneal diseases are among the major causes of visual impairment and blindness. In clinic, noninvasive corneal imaging plays a vital role in the diagnosis of corneal diseases. However, current imaging devices still couldn't achieve the spatial information of cornea at cellular or subcellular level. The purpose of this study is to evaluate the capacity of Micro optical coherence tomography (μOCT) in detecting cellular or subcellular structures of rat cornea both ex vivo and in vivo. For ex vivo study, eyes of normal adult rats were enucleated immediately after euthanasia and were placed into a container with the corneal side up for image acquisition. Corneal layers including epithelium, stroma, Descemet' s membrane (DM) and endothelium were clearly differentiated in cross-sectional views which is comparable to histology. En face images reconstructed from 3D images at different depths present a good quality of cellular microstructures including hexagonally shaped cells in endothelium, high scattering lattice at the interface of endothelium and DM, and dendritic keratocytes and linear collagen fibers from posterior and anterior stroma. For in vivo study, a rat was mounted onto the scanning stage of μOCT system after anesthetized. Microstructures of cornea were also visible on the three dimensional images captured by μOCT. Our results indicate that μOCT is able to visualize cellular components of cornea both in vivo and ex vivo and it will assist our understanding of cornea and the diagnosis of corneal diseases.

10045-72, Session PSun

Concurrent OCT imaging of stimulus evoked retinal neural activation and hemodynamic response

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It is well established that major retinal diseases involve distortions of retinal neural physiology and blood vascular system. However, details of distortions in retinal neurovascular coupling associated with these major eye diseases are not well understood. In this study, a multi-modal optical coherence tomography (OCT) imaging system was developed to enable concurrent imaging of retinal neural activity and vascular hemodynamics. Flicker light stimulation was applied to mouse retinas to evoke retinal neural responses and hemodynamic changes. The OCT images were acquired continuously during pre-stimulation phase, light-stimulation phase and post-stimulation phase. Stimulus- evoked intrinsic optical signal (IOS) and hemodynamic changes were observed over time in blood-free and blood regions, respectively. The IOS changes showed almost immediately onset after the stimulation. Both positive and negative signals were observed in similar degree. The hemodynamic changes showed somewhat delayed onset about 5-7 second after stimulation and positive signal changes were relatively larger than negative signal. The signal magnitudes induced by light stimulation were larger in blood regions than that in blood free regions. The response from blood regions and blood-free regions showed somewhat different changes. These differences may come from different mechanism on light stimulation between blood vessel and neural tissue. These characteristics agree well with our previous results from mouse retina. Further development of the multi-modal OCT may provide a new imaging methodology to study how retinal structure, metabolic and neural functions affected by age-related macular degeneration (AMD), glaucoma, diabetic retinopathy (DR), etc., promising noninvasive biomarkers for early disease detection and reliable treatment evaluation.

10045-73, Session PSun

Measurement of the human eye axes and angles using the whole-eye OCT

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Human eyes have evolved to have three axes: the visual axis (VA), the optical axis (OA), and the pupillary axis (PA). These axes have two angles of alpha and kappa formed by the intersection of the VA with the OA and the VA with the PA, respectively. The three axes and the two angels play an important role for refractive surgery, especially for the cataract surgery and the LASIK surgery. The traditional method for measuring the axes and the angles, however, do not provide accurate values because the inspection is limited only to the surface of the corneal region, which leads to unexpected side effects for patients. In this study, we propose a new method to define the three axes and the two angles of human eyes more quantitatively using optical coherence tomography (OCT). This OCT system can measure an anterior segment and a retina image simultaneously and thus is called the whole-eye OCT. The whole-eye OCT uses two orthogonally polarized beams from a swept-source and the polarized beams are focused on an anterior segment and a retina one for each. A whole-eye image is reconstructed and rescaled accounting for the refractive indices of the structures of the human eye from the images of the anterior segment and the retina. The three axes, as well as the two angles of the eyes, are then measured using the reconstructed whole-eye image by the definition. Our method will facilitate the precise measurement of the geometric parameters characterizing the shape of human eyes.

10045-74, Session PSun

Noncontact optical coherence elastography of the posterior porcine sclera in situ as a function of IOP

Manmohan Singh, Achuth Nair, Univ. of Houston (United States); Salavat R. Aglyamov, The Univ. of Texas at Austin (United States); Chen Wu, Zhaolong Han, Ericka Lafon, Univ. of Houston (United States); Kirill V. Larin, Univ. of Houston (United States) and Baylor College of Medicine (United States)

Recent work has shown that the biomechanical properties of tissues in the posterior eye have are critical for understanding the etiology and progression of ocular diseases. For instance, the primary risk for glaucoma is an elevated intraocular pressure (IOP). Weak tissues will deform under the large pressure, causing damage to vital tissues. In addition, scleral elasticity can influence the shape of the eye-globe, altering the axial length. In this work, we utilize a noncontact form of optical coherence elastography (OCE) to quantify the spatial distribution of biomechanical properties of the optic nerve, its surrounding tissues, and posterior sclera on the exterior of in situ porcine eyes in the whole eye-globe configuration. The OCE measurements were taken at various IOPs to evaluate the biomechanical properties of the tissues as a function of IOP. The air-pulse induced dynamic response of the tissues was translated to Young's modulus by a model-based viscoelasticity reconstruction technique. The results show that the posterior sclera is not as stiff as the optic nerve and its surrounding tissues (~6 kPa and ~ 13 kPa at 10 mmHg IOP, respectively). Moreover, the scleral stiffness was unaffected by IOP (~6 kPa at 10 mmHg IOP to ~9 kPa at 20 mmHg), whereas the optic nerve and its surrounding tissues were stiffened as IOP was increased (~13 kPa at 10 mmHg to ~19 kPa at 20 mmHg).

10045-75, Session PSun

Longitudinal visualization of vascular occlusion, reperfusion, and remodeling in a zebrafish model of retinal vascular leakage using OCT angiography

Kathleen Spitz, Ivan Bozic, Vanderbilt Univ. (United States); Brent A. Bell, Rose DiCicco, Lana M. Pollock, Bela Anand-Apte, The Cleveland Clinic (United States); Yuankai K. Tao, Vanderbilt Univ. (United States)

Diabetic retinopathy (DR) and age-related macular degeneration (AMD) are two of the leading causes of blindness and visual impairment in the world. Neovascularization results in severe vision loss in DR and AMD and, thus, there is an unmet need to identify mechanisms of pathogenesis and novel anti-angiogenic therapies. Zebrafish is a leading model organism for studying human disease pathogenesis, and the highly conserved drug activity between zebrafish and humans and their ability to readily absorb small molecules dissolved in water has benefited pharmaceutical discovery. Here, we use optical coherence tomography (OCT) and OCT angiography (OCT-A) to perform noninvasive, in vivo retinal imaging in a zebrafish model of vascular leakage. Zebrafish were treated with diethylaminobenzaldehyde (DEAB) to induce vascular leakage and imaged with OCT and OCT-A at six time points over two weeks: baseline one day before treatment and one, three, six, eight, and ten days post treatment. Longitudinal functional imaging showed significant vascular response immediately after DEAB treatment. Observed vascular changes included partial or complete vascular occlusion immediately after treatment and reperfusion during a two-week period. Increased vascular tortuosity several days post treatment indicated remodeling, and bifurcations and collateral vessel formation were also observed. In addition, significant treatment response variabilities were observed in the contralateral eye of the same animal. Anatomical and functional normalization was observed in most animals by ten days post treatment. These preliminary results motivate potential applications of OCT-A as a tool for studying pathogenesis and therapeutic screening in zebrafish models of retinal vascular disease.

Conference 10046: Visualizing and Quantifying Drug Distribution in Tissue

Saturday 28–28 January 2017

Part of Proceedings of SPIE Vol. 10046 Visualizing and Quantifying Drug Distribution in Tissue

10046-1, Session 1

Imaging and quantifying drugs with coherent Raman scattering *(Invited Paper)*

Conor L. Evans, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States)

Coherent Raman scattering (CRS) microscopies offer the ability to carry out molecular imaging of drugs, actives, and cosmetics based on the molecular vibrations unique to their chemical structures. Unlike fluorescent labeling approaches that can alter the uptake and distribution of drugs, CRS tools enable tracking and quantification of unperturbed molecules. Coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) microscopies have found many applications since first demonstrated in vivo twelve years ago, ranging from following the diffusion of oils in skin to tracking the uptake of small molecule tyrosine kinase inhibitors. Vibrational labeling strategies, where molecules are synthesized to contain Raman labels, have opened the ability to visualize a wide range of molecules through techniques including deuterium isotope labeling and the introduction of nitrile groups. With these methods, CRS imaging can be used to track, in real-time, the uptake and distribution of drugs in tissue, making it an important new tool for drug development.

10046-2, Session 1

Imaging subcellular distribution of ferroptosis inhibitor in living cells with stimulated Raman scattering microscopy

Fanghao Hu, Michael M. Gaschler, Brent R. Stockwell, Wei Min, Columbia Univ. (United States)

Ferroptosis is an oxidative, iron-dependent form of non-apoptotic cell death. Small molecule ferrostatins are found to protect against ferroptosis by its antioxidant activity. The exact mechanism of action of ferrostatin is unknown. By labeling with a small vibrational tag, the ferrostatin derivative is shown to have comparable potency and its subcellular localization is visualized by stimulated Raman scattering microscopy with high spatial and temporal resolution. This can help elucidating the kinetics and place of action of ferrostatin inside cells and provide mechanistic insight into ferroptosis.

10046-3, Session 1

Towards noninvasive assessment of drug distribution in tissue: coherent Raman scattering from chiral molecules

Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Many biologically active molecules are chiral. Many drugs, which are currently in use, are supplied as an equimolar mixture of two enantiomers. While they have the same chemical structure, i.e. not distinguishable by conventional Raman spectroscopy, most isomers of chiral drugs exhibit marked differences in biological activities such as pharmacology, toxicology, pharmacokinetics, and metabolism. In this report we introduce a new spectroscopic tool to extend nonlinear Raman scattering to chiral substances. As a proof of principle, we demonstrated detection of D-glucose at physiologically relevant concentrations.

10046-4, Session 1

Noninvasive label-free monitoring of cosmetics and pharmaceuticals in human skin using nonlinear optical microscopy

Sam Osseiran, Hequn Wang, Conor L. Evans, Massachusetts General Hospital (United States)

Over the past decade, nonlinear optical microscopy has seen a dramatic rise in its use in research settings due to its noninvasiveness, enhanced penetration depth, intrinsic optical sectioning, and the ability to probe chemical compounds with molecular specificity without exogenous contrast agents. Nonlinear optical techniques including two-photon excitation fluorescence (2PEF), fluorescence lifetime imaging microscopy (FLIM), second harmonic generation (SHG), coherent anti-Stokes and stimulated Raman scattering (CARS and SRS, respectively), as well as transient and sum frequency absorption (TA and SFA, respectively), have been widely used to explore the physiology and microanatomy of skin. Recently, these modalities have shed light on dermal processes that could not have otherwise been observed, including the spatiotemporal monitoring of cosmetics and pharmaceuticals. However, a challenge quickly arises when studying such chemicals in a dermatological context: many exogenous compounds have optical signatures that can interfere with the signals that would otherwise be acquired from intact skin. For example, oily solvents exhibit strong signals when probing CH2 vibrations with CARS/SRS; chemical sun filters appear bright in 2PEF microscopy; and darkly colored compounds readily absorb light across a broad spectrum, producing strong TA/SFA signals. Thus, this discussion will first focus on the molecular contrast in skin that can be probed using the aforementioned nonlinear optical techniques. This will be followed by an overview of strategies that take advantage of the exogenous compounds' optical signatures to probe spatiotemporal dynamics while preserving endogenous information from skin.

10046-5, Session 1

Surface-enhanced Raman scattering (SERS) imaging of alkyne-tagged small molecule drug in live cells with endocytosed gold nanoparticles

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Raman scattering microscopy can directly visualize drug distribution in biological sample by observing the vibrational signature of the target molecule. However, inherent low sensitivity of Raman spectroscopy and complex background Raman signal from endogenous biomolecules often prevents the analysis. Here, we propose the combination of surface-enhanced Raman scattering (SERS) spectroscopy and alkyne-tag to monitor drugs in cells with high sensitivity and selectivity. Gold nanoparticles, which was incubated with living HeLa cells, were incorporated in cytoplasm by endocytosis, and accumulated in lysosome by cellular transport pathways. The endocytosed nanoparticles work as SERS agent to amplify Raman signal from target molecules that enter in the lysosomal environment. We then

administrated alkyne-tagged inhibitor of lysosomal enzyme in cell culture medium. The alkyne shows distinct peak at Raman-silent region of biomolecules. Therefore, selective detection of tagged molecule becomes possible without the overlap of Raman signal from endogenous molecules. Since the alkyne has tiny chemical structure, it has minimum influence

on original property of the small molecule. We used slit-scanning Raman microscopy with 676 nm excitation laser. Exposure time was set as 1 second per line for imaging. After the administration of alkyne-tagged inhibitor, we observed the strong Raman signal of alkyne from endocytosed nanoparticles in living HeLa cells, indicating the arrival of the inhibitor in lysosome.

10046-6, Session 1

Advanced imaging approaches for characterizing nanoparticle delivery and dispersion in skin

Tarl W. Prow, Miko Yamada, Nhung Dang, The Univ. of Queensland (Australia); Conor L. Evans, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States)

The purpose of this research was to develop advanced imaging approaches to characterise the combination of elongated silica microparticles (EMP) and nanoparticles to control topical delivery of drugs and peptides. The microparticles penetrate through the epidermis and stop at the dermal-epidermal junction (DEJ). In this study we incorporated a fluorescent lipophilic dye, DiI, as a hydrophobic drug surrogate into the nanoparticle for visualization with microscopy. In another nanoparticle-based approach we utilized a chemically functionalized melanin nanoparticle for peptide delivery. These nanoparticles were imaged by coherent anti-Stoke Raman scattering (CARS) microscopy to characterize the delivery of these nanoparticles into freshly excised human skin. We compared four different coating approaches to combine EMP and nanoparticles. These data showed that a freeze-dried formulation with cross-linked alginate resulted in 100% of the detectable nanoparticle retained on the EMP. When this dry form of EMP-nanoparticle was applied to excised, living human abdominal skin, the EMP penetrated to the DEJ followed by controlled release of the nanoparticles. This formulation resulted in a sustained release profile, whereas a freeze-dried formulation without crosslinking showed an immediate burst-type release profile. These data show that advanced imaging techniques can give unique, label free data that shows promise for clinical investigations.

10046-7, Session 2

Combining imaging with microbiopsy enables a more comprehensive approach for topical drug research *(Invited Paper)*

Tarl W. Prow, The Univ. of Queensland (Australia)

Topical drug delivery is a challenging research field, but quantifying topical drug delivery also has significant challenges, especially in the clinical studies. Both cosmeceutical and pharmaceutical endpoints largely drive research in this area. Conventional drug delivery approaches primarily rely on testing trans dermal drug kinetics using excised skin in Franz cells. Thus is a largely unmet need for non- and minimally invasive approaches to evaluate topical drug delivery and efficacy in excised and volunteer skin. We are meeting this need through the development of non-invasive imaging based approaches such as fluorescent dermoscopy, fluorescence scanning and confocal microscopy followed by image analysis. Minimally invasive microbiopsies are being used to extract drug concentrations from tiny pieces of skin without the need for local anaesthetics and without scars. This combined strategy enables us to collect drug disposition information in addition to skin morphology and molecular characterisation which provides a more dynamic and comprehensive way to examine drug deliver, effects of enhancement technologies and efficacy.

10046-8, Session 2

Targeting tumour with theranostic nanoconstructs for selective PDT *(Invited Paper)*

Huang Chiao Huang, Joyce Liu, Sriram R. Anbil, Girgis Obaid, Imran Rizvi, Tayyaba Hasan, Massachusetts General Hospital (United States)

Photodynamic therapy (PDT) is a photochemistry-based modality that involves a combination of non-cytotoxic photosensitizers and visible light to generate cytotoxic molecular species, modulating and killing malignant cells and tissues. The photosensitizers can also serve as fluorescence imaging agents and have been useful in diagnostic applications, image-guided surgery and customized PDT dosimetry. In addition to the spatial selectivity conferred by focused light irradiation, a broad range of targeting methods have amplified the effectiveness of PDT by reducing the off-target distribution of photosensitizers to healthy tissue. Here, we report activatable theranostic nanoconstructs that exploit three sub-sets of tumour targeting mechanisms to achieve selective PDT. These mechanisms include (1) 'targeted delivery' of photosensitizers and drugs through the recognition of tumor-associated antigens, (2) targeting through 'tissue (microvasculature and stromal) modulation' to enhance PDT and drug delivery efficacy, and (3) 'functional targeting' that utilizes the physical and subcellular biochemical processes of tumours. As we continue to understand tumorigenesis, progression and therapeutic resistance, advances in optical technologies combined with targeted nanoconstructs containing photosensitizers offer new prospects to selectively apply personalized medicine for cancer and other diseases.

10046-9, Session 2

Visualization of drug distribution of topical minocycline in human facial skin with fluorescence microscopy

Maiko Hermsmeier, Tanvee Sawant, Diana Lac, Akira Yamamoto, Xin Chen, Usha Nagavarapu, BioPharmX, Inc. (United States); Conor L. Evans, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Kin Foong Chan, BioPharmX, Inc. (United States)

Minocycline is an antibiotic regularly prescribed to treat acne vulgaris. The only commercially available minocycline comes in an oral dosage form, which often results in systemic adverse effects. A topical minocycline composition (BPX-01) was developed to provide localized and targeted delivery to the epidermis and pilosebaceous unit where acne-related bacteria, Propionibacterium acnes (P. acnes), reside. As minocycline is a known fluorophore, fluorescence microscopy was performed to investigate its potential use in visualizing minocycline distribution within tissues. BPX-01 with various concentrations of minocycline, was applied topically to freshly excised human facial skin specimens. Spatial distribution of minocycline and its fluorescence intensity within the stratum corneum, epidermis, dermis, and pilosebaceous unit were assessed. The resulting fluorescence intensity data as a function of minocycline concentration may indicate clinically relevant therapeutic doses of topical BPX-01 needed to kill P. acnes and reduce inflammation for successful clinical outcomes.

10046-10, Session 2

Quantitative imaging of intracellular signaling for personalized pancreatic cancer therapy in an in ovo avatar

Kimberley S. Samkoe, Geisel School of Medicine,

Dartmouth College (United States) and Dartmouth College (United States); Emily Schultz, Oregon Health & Science Univ. (United States); Yeonjae Park, Dartmouth College (United States); Dawn Fischer, Geisel School of Medicine, Dartmouth College (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States); Kerrington Smith, Geisel School of Medicine, Dartmouth College (United States); Kenneth M. Tichauer, Illinois Institute of Technology (United States); Summer L. Gibbs, Oregon Health & Science Univ. (United States)

Pancreatic ductal adenocarcinomas (PDAC) are notoriously difficult to treat and in general, molecular targeted therapies have failed even when the targeted protein is overexpressed in the tumor tissue. Genetic mutations in extracellular receptors and downstream signaling proteins (i.e., RAS signaling pathway) and convoluted intracellular cross-talk between cell signaling pathways are likely reasons that these promising therapies fail. Monitoring the complex relationship between intracellular protein signaling is difficult and to-date, standard techniques that are used (Western blot, flow cytometry, immunohistochemistry, etc.) are invasive, static and do not accurately represent in vivo structure-function relationships. Here, we describe the development of an in ovo avatar using patient derived tumors grown on the chicken chorioallantoic membrane (CAM) and the novel fluorescence-based Quantitative Protein Expression Tracking (QUIET) methodology to bridge the gap between oncology, genomics and patient outcomes. Previously developed paired-agent imaging, was extended to a three-compartment model system in QUIET, which utilizes three types of imaging agents: novel fluorophore conjugated cell permeable targeted and untargeted small molecule paired-agents, in addition to a tumor perfusion agent that is not cell membrane permeable. We have demonstrated the ability to quantify the intracellular binding domain of a trans-membrane protein in vitro using cell permeable fluorescent agents (erlotinib-TRITC and control isotype-BODIPY FL). In addition, we have demonstrated imaging protocols to simultaneously image up to 6 spectrally distinct organic fluorophores in in ovo avatars using the Nuance EX (Perkin Elmer) and established proof-of-principle intracellular and extracellular protein concentrations of epidermal growth factor receptor using QUIET and traditional paired-agent imaging.

10046-11, Session 3

Direct visualization of functional heterogeneity in hepatobiliary metabolism *(Invited Paper)*

Chih-Ju Lin, Feng-Chieh Li, Yu-Yang Lee, Te-Yu Tseng, Wei-Liang Chen, Vladimir A. Hovhannisyan, Ning Kang, National Taiwan Univ. (Taiwan); Nicholas G. Horton, Cornell Univ. (United States); Shu-Jen Chiang, National Taiwan Univ. (Taiwan); Chris Xu, Cornell Univ. (United States); Hsuan-Shu Lee M.D., National Taiwan Univ. Hospital (Taiwan); Chen-Yuan Dong, National Taiwan Univ. (Taiwan)

Metabolism is one of the major functions of the hepatobiliary system. However, little is known of the relationship between physiological location of the hepatocytes and their metabolic efficacy. Since liver is a major organ in the processing of drug molecules, clarification of how different part of the liver process molecules is potentially important for optimal delivery of therapeutic agents.By combining time-lapse multiphoton microscopyand first order kinetic constant image analysis, hepatocellular metabolic rate is quantified at single cell level, using 6-CFDA as model compound.We found that the mouse liver can be divided into three zones, each with distinct metabolic rate constants. Our results show the existence of heterogeneities in hepatobilitary metabolism and that Zone 3 is the main area of hepatic acinus responsible in metabolim.

10046-12, Session 3

Comparison of corneal riboflavin gradients using dextran and HPMC solutions

Tobias Ehmke, Laser Zentrum Hannover e.V. (Germany); Theo G. Seiler, Isaak Fischinger, Inselspital, Univ. Bern (Switzerland) and IROC AG (Switzerland); Tammo Ripken, Laser Zentrum Hannover e.V. (Germany); Beatrice E. Frueh, Inselspital, Univ. Bern (Switzerland); Alexander Heisterkamp, Leibniz Univ. Hannover (Germany) and Laser Zentrum Hannover e.V. (Germany)

Corneal crosslinking is considered to be a safe and effective procedure to halt the progression of keratoconus. In order to create corneal crosslinks, the Dresden protocol is often used in clinical routines: after removing the epithelium, riboflavin dissolved in aqueous 20% dextran solution is applied to the surface of the cornea which leads to diffusion of riboflavin into the corneal stroma. Subsequent UV(A) irradiation activates the riboflavin and results in formation of crosslinks. The whole procedure takes 60 minutes. Since this is a very long time for both patient and physician it is desirable to shorten the treatment time. One approach is to use other solvents of riboflavin like hydroxyl propyl methyl cellulose (HPMC) instead of dextran. In this work, the riboflavin concentration gradient in the anterior corneal stroma when using HPMC or dextran as carrier agent was determined. Four different groups of porcine corneas were compared regarding the riboflavin concentration in the anterior stroma. Groups 1 and 2 were treated with 0.1% riboflavin in 20% dextran for 10 and 30 minutes, groups 3 and 4 with 0.1% riboflavin in 1.1% HPMC for 10 and 30 minutes. After imbibition, multiphoton microscopy was used to determine two-photon fluorescence intensity. A correction of these intensity data in consideration of scattering and absorption effects allowed the determination of riboflavin concentrations. Comparing groups 2 and 3, a significant higher stromal riboflavin concentration was found within the anterior 70 ?m in the dextran group, whereas deeper than 260 ?m HPMC assisted imbibition yielded higher concentrations.

10046-13, Session 3

Imaging bio-distribution of a topically applied dermatological cream on minipig skin using fluorescence lifetime imaging microscopy

Aneesh Alex, GlaxoSmithKline (United States); Eric J. Chaney, Jennifer M. Criley, Darold R. Spillman Jr., Phaedra B. Hutchison, Joanne Li, Marina Marjanovic, Univ. of Illinois at Urbana-Champaign (United States); Steve Frey, Steven Cook, GlaxoSmithKline (United States); Stephen A. Boppart, Univ. of Illinois at Urbana-Champaign (United States); Zane A. Arp, GlaxoSmithKline (United States)

Currently there is a lack of in vivo techniques to evaluate the spatial bio-distribution of dermal drugs over time without the need to take multiple serial biopsies. To address this gap, we investigated the use of multi-photon optical imaging methods to non-invasively track drug distribution on miniature pig (Species: Sus scrofa, Strain: Göttingen) skin in vivo. Minipig skin is the standard comparative research model to human skin, and is anatomically and functionally similar. We employed fluorescence lifetime imaging microscopy (FLIM) to visualize the spatial distribution and residency time of a topically applied experimental dermatological cream. This was made possible by the endogenous fluorescent optical properties of the experimental drug (fluorescence lifetime > 3000 ps). Two different drug formulations were applied on 2 minipigs for 7 consecutive days, with the control creams applied on the contralateral side, followed by 7 days of post-application monitoring using a multi-modal optical imaging system (MPTflex-CARS, JenLab, Germany). FLIM images were obtained from the

treated regions 24 hr post-application from day 1 to day 14 that allowed visualization of cellular and sub-cellular features associated with different dermal layers non-invasively to a depth of 200 μm. Five punch biopsies per animal were obtained from the corresponding treated regions between days 8 and 14 for bioanalytical analysis and comparison with results obtained using FLIM. In conclusion, utilization of non-invasive optical biopsy methods for dermal drug evaluation can provide true longitudinal monitoring of drug spatial distribution, remove sampling limitations, and be more time-efficient compared to traditional methods.

10046-14, Session 4

Mass spectrometry imaging applications to characterize and quantify drug distribution in tissue from animal models and clinical trials *(Invited Paper)*

Nathalie Agar, Brigham and Women's Hospital (United States)

An effective targeted therapeutic must both meet criteria for potency, and reach cancer cells at therapeutic levels. In solid tumors of the brain, reaching therapeutic drug levels is limited by the blood-brain barrier, but detailed characterization of drug distribution in the brain has been limited by the lack of tools to directly image small molecules without altering their chemistry with the use of molecular probes. Matrix Assisted Laser Desorption Ionization (MALDI) Fourier Transform Ion Cyclotron Resonance (FTICR) Mass Spectrometry Imaging (MSI) can now be used to characterize and quantitate the distribution of intact small molecules and their metabolites in tissue.

10046-15, Session 4

Identification and localization of trauma-related biomarkers using matrix assisted laser desorption/ionization imaging mass spectrometry

Kirstin Jones, The Univ. of Texas Health Science Ctr. at San Antonio (United States); Matthew A. Reilly, The Ohio State Univ. (United States); Randolph D. Glickman, The Univ. of Texas Health Science Ctr. at San Antonio (United States)

Current treatments for traumatic ocular and optic nerve neuropathy are largely ineffective and may have adverse side effects; therefore, new methods to characterize ocular tissue responses following traumatic injuries are needed. Identification of trauma-related biomarkers is a promising approach for investigating the molecular aspects of tissue trauma, which can contribute to the development of better trauma treatments. The conventional approach for protein biomarker measurement largely relies on immunoaffinity methods, such as ELISA and bead-based, multianalyte assays. Matrix assisted laser-assisted desorption/ionization imaging mass spectrometry (MALDI IMS) is a specialized application of mass spectrometry that not only is well suited to the discovery of novel or unanticipated biomarkers, but also provides information about the spatial localization of biomarkers in tissue. We have been using MALDI IMS to find trauma-related protein biomarkers in retina and optic nerve tissue from rats subjected to traumatic optic neuropathy induced by a programmable robotic system. Work to date by our group, using MALDI IMS, suggested that the pattern of protein expression is modified in the injured optic nerves at 7 days post-injury, compared to controls, with a decrease in the height of specific protein peaks in the injured tissue. This indicates lower expression or perhaps modification of particular proteins resulting from the tissue response to the injury. Ongoing work is directed at identifying the proteins affected and mapping their expression in the ocular tissue. Ultimately, this type of systematic analysis may help develop improved diagnostics or assess the effect of prospective therapies for ocular trauma.

10046-16, Session 4

Miniaturizing 3D assay for high-throughput drug and genetic screens for small patient-derived tumor samples *(Invited Paper)*

Asaf Rotem, Levi Garraway, Mei-Ju Su, Dana-Farber/ Harvard Cancer Ctr. (United States); Anindita Basu, Aviv Regev, Broad Institute of MIT and Harvard (United States); Kevin Struhl, Harvard Medical School (United States)

Three-dimensional growth conditions reflect the natural environment of cancer cells and are crucial to be performed at drug screens. We developed a 3D assay for cellular transformation that involves growth in low attachment (GILA) conditions and is strongly correlated with the 50-year old benchmark assay-soft agar. Using GILA, we performed high-throughput screens for drugs and genes that selectively inhibit or increase transformation, but not proliferation.

This phenotypic approach is complementary to our genetic approach that utilizes single-cell RNA-sequencing of a patient sample to identify putative oncogenes that confer sensitivity to drugs designed to specifically inhibit the identified oncoprotein.

Currently, we are dealing with a big challenge in our field- the limited number of cells that might be extracted from a biopsy. Small patient-derived samples are hard to test in the traditional multiwell plate and it will be helpful to minimize the culture area and the experimental system. We managed to design a suitable microfluidic device for limited number of cells and perform the assay using image analysis.

We aim to test drugs on tumor cells, outside of the patient body- and recommend on the ideal treatment that is tailored to the individual. This device will help to minimize biopsy-sampling volumes and minimize interventions in the patient's tumor.

10046-17, Session 4

In vivo optical coherence tomography imaging of penetration and dissolution characteristics of hyaluronic acid microneedles in human skin

Seungri Song, Yonsei Univ. (Korea, Republic of); Jung Dong Kim, Jung-hyun Bae, Raphas Co., Ltd. (Korea, Republic of); Sooho Chang, Soocheol Kim, Hyungsuk Lee, Yonsei Univ. (Korea, Republic of); Dohyeon Jeong, Raphas Co., Ltd (Korea, Republic of); Hong Kee Kim, Raphas Co., Ltd. (Korea, Republic of); Chulmin Joo, Yonsei Univ. (Korea, Republic of)

Transdermal drug delivery (TDD) has been recently highlighted as an alternative to oral delivery and hypodermic injections. Among many methods, drug delivery using a microneedle (MN) is one of the promising administration strategies due to its high skin permeability, mininal invasiveness, and ease of injection. In addition, microneedle-based TDD is explored for cosmetic and therapeutic purposes, rapidly developing market of microneedle industry for general population.

To date, visualization of microneedles inserted into biological tissue has primarily been performed ex vivo. MRI, CT and ultrasound imaging do not provide sufficient spatial resolution, and optical microscopy is not suitable because of their limited imaging depth; structure of microneedles located in 0.2-1mm into the skin cannot be visulalized.

Optical coherence tomography (OCT) is a non-invasive, cross-sectional optical imaging modality for biological tissue with high spatial resolution and acquisition speed. Compared with ultrasound imaging, it exhibits superior spatial resolution (1-10 um) and high sensitivity, while providing an imaging depth of biological tissue down to 1-2 mm. Here, we present in situ

imaging and analysis of the penetration and dissolution characteristics of hyaluronic acid based MNs (HA-MN) with various needle heights in human skin in vivo. In contrast to other studies, we measured the actual penetration depths of the HA-MNs by considering the experimentally measured refractive index of HA in the solid state. For the dissolution dynamics of the HA-MNs, time-lapse structural alteration of the MNs could be clearly visualized, and the volumetric changes of the MNs were measured with an image analysis algorithm.

10046-18, Session 4

Label-free measurement of spatiotemporal drug distributions in mucosal tissue with co-localized confocal Raman spectroscopy and optical coherence tomography (CRS-OCT)

Jason R. Maher, Oranat Chuchuen, Michael DeSoto, Marcus H. Henderson, Adam Wax, David F. Katz, Duke Univ. (United States)

Prophylactic and therapeutic drugs may act topically, and must be delivered to local tissue sites. In order to improve the efficacy of many such drugs, a better understanding of their transport and delivery processes is needed. Unfortunately, current methodologies to measure drug distributions in tissue are often invasive, expensive, slow, and/or labor intensive.

Optical imaging and spectroscopy methods offer an attractive alternative for measuring drug transport in tissue. For example, confocal Raman spectroscopy (CRS) can measure spatiotemporal distributions of a wide range of analytes in tissue. Imaging modalities such as optical coherence tomography (OCT) can be used to guide these measurements by providing high-resolution, cross-sectional images of tissue morphology.

In this talk, we present a multimodal CRS-OCT instrument capable of measuring analytes in targeted biological tissues with sub-50-micron spatial resolution. Our work has focused primarily on topical microbicide drugs delivered to mucosa to prevent sexual transmission of HIV. Recently, we developed an assay to measure spatiotemporal distributions of the microbicide drug Tenofovir in porcine vaginal tissue. Fundamental transport parameters, diffusion coefficients in epithelium and stroma, and the partition coefficient between those layers, were determined by fitting data with a compartmental diffusion model. The results indicate that Tenofovir diffusion is slower in the epithelium, and that this layer is rate limiting for drug transport into the underlying stromal tissue where it acts against HIV. Future applications of this assay can guide the development of microbicides as well as a variety of other topically acting drugs.

10046-19, Session 4

Beyond autoradiography: innovations in preclinical tissue distribution methods to solve problems in drug discovery and development

Marissa Vavrek, Carol Freddo, Carol Kertesz, Merck Research Labs. (United States); Gary Van Berkel, Oak Ridge National Lab. (United States)

Quantitative whole body autoradiography (QWBA) using radiolabeled compounds is the gold standard for preclinical tissue distribution studies, providing accurate and reliable quantification of total drug related material (DRM) in tissues. The inherent non-selectivity of QWBA and inability to resolve cellular disposition provide opportunities for innovative pairings with other methodologies. Major advances have been made in mass spectrometric imaging and surface sampling techniques for molecular identification of DRM in tissue samples. In addition, higher resolution methodologies including microautoradiography (MARG), transmission electron microscopy, and nano-secondary ion mass spectrometry (nanoSIMS) present unique opportunities for studying various therapeutic agents at the cellular and subcellular level. Examples highlighting pairing of more traditional QWBA methodologies with mass spectrometry, MARG, and nanoSIMS will be highlighted.

Conference 10047: Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXVI

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10047-1, Session 1

Determinants of photokilling by mitochondrial vs lysosomal photodamage *(Invited Paper)*

David H. Kessel, Wayne State Univ. School of Medicine (United States)

In 1996, it was reported that a sequential PDT protocol involving lysosomal photodamage (LPDT) followed by PDT targeting mitochondria (MPDT) could promote eradication of large sarcomas in a mouse model. Neither process alone showed significant cancer control regardless of the PDT dose. We have now shown that this process involves an initial opening of calcium channels in lysosomes, leading to activation of calpain and subsequent cleavage of the autophagy-associated protein ATG5 to a truncated product (tATG5) that promotes apoptosis initiated by mitochondral photodamage. Since tATG5 is unstable, MPDT needs to promptly follow LPDT. It is also feasible to simultaneously create photodamage at both subcellular sites using a broad-band light source with no loss in photokilling. It is perhaps noteworthy that Photofrin can initiate both LPDT and MPDT, a possible explanation for the efficacy of an agent with only a weak absorbance at a sub-optimal wavelength..

10047-2, Session 1

Repurposing of photodynamic therapy (PDT) and established drugs in cancer therapeutics *(Invited Paper)*

Tayyaba Hasan, Wellman Ctr. for Photomedicine (United States)

Considering the consistently poor prognoses for some of the deadliest cancers, as well as the skyrocketing costs (~$1-2 billion) and long time frame (~12-16 years) for developing a brand-new drug, rapidly translatable agents that offer improvements in outcomes are much needed. Drug repurposing is one such strategy to decrease costs, reduce time frame to clinical translation, and possibly increase success rates. This presentation will elucidate the benefit of this approach with drugs like the tetracyclines (a class of antibiotics), vitamins and chemotherapeutics combined with PDT to overcome chemoresistance in pancreatic and ovarian cancers.

10047-7, Session 1

Therapeutic enhancement of aminolevulinic acid-based tumor therapy

Bin Chen, Univ. of the Sciences in Philadelphia (United States)

Photodynamic therapy (PDT) involves the combination of a photosensitizer and light of a specific wavelength. Upon light activation in the presence of oxygen, photosensitizer molecules generate reactive oxygen species that cause cytotoxicity by inducing oxidative stress. Aminolevulinic acid (ALA) is a pro-drug used for the diagnosis and PDT treatment of various solid tumors based on endogenous production of heme precursor protoporphyrin IX (PpIX). Although nearly all types of human cells express heme biosynthesis enzymes and produce PpIX, tumor cells are found to have more PpIX production and accumulation than normal cells, allowing for the detection and treatment of solid tumors.

The objective of my research is to explore therapeutic approaches to enhance ALA-based tumor detection and therapy. We have found that high ABCG2 transporter activity in triple negative breast cancer cells (TNBC) contributed to reduced PpIX levels in cells, causing them to be more resistant towards ALA-PDT. The administration of an ABCG2 inhibitor, Ko143, was able to reverse cell resistance to ALA-PDT by enhancing PpIX mitochondrial accumulation and sensitizing cancer cells to ALA-PDT. Ko143 treatment had little effect on PpIX production and ALA-PDT in normal and ER- or HER2-positive cells. Furthermore, since some tyrosine kinase inhibitors (TKI) are known to block ABCG2 transporter activity, we screened a panel of tyrosine kinase inhibitors to examine its effect on enhancing PpIX fluorescence and ALA-PDT efficacy. Several TKIs including lapatinib and gefitinib showed effectiveness in increasing ALA-PpIX fluorescence in TNBC leading to increased cell death after PDT administration. These results indicate that inhibiting ABCG2 transporter using TKIs is a promising approach for targeting TNBC with ALA-based modality.

10047-3, Session 2

Molecular and cellular mechanisms of PDT-based combinations in overcoming chemoresistance from heterotypic cellular communication and physical stress in 3D cancer models *(Invited Paper)*

Imran Rizvi, Anne-Laure Bulin, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Sriram R. Anbil, Howard Hughes Medical Institute (United States) and Univ. of Texas School of Medicine in San Antonio (United States) and Harvard Medical School (United States); Emma A. Briars, Massachusetts General Hospital (United States); Daniela Vecchio, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Jonathan P. Celli, Univ. of Massachusetts Boston (United States); Mans Broekgaarden, Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

Targeting the molecular and cellular cues that influence treatment resistance in tumors is critical to effectively treating unresponsive populations of stubborn disease. The informed design of mechanism-based combinations is emerging as increasingly important to targeting resistance and improving the efficacy of conventional treatments, while minimizing toxicity. Photodynamic therapy (PDT) has been shown to synergize with conventional agents and to overcome the evasion pathways that cause resistance. Increasing evidence shows that PDT-based combinations cooperate mechanistically with, and improve the therapeutic index of, traditional chemotherapies. These and other findings emphasize the importance of including PDT as part of comprehensive treatment plans for cancer, particularly in complex disease sites. Identifying effective combinations requires a multi-faceted approach that includes the development of bioengineered cancer models and corresponding image analysis tools. The molecular and phenotypic basis of verteporfin-mediated PDT-based enhancement of chemotherapeutic efficacy and predictability in complex 3D models for ovarian cancer will be presented.

10047-4, Session 2

Impact of photodynamic therapy on extracellular matrix components (and vice versa) in 3D pancreatic tumor models *(Invited Paper)*

Seyedehrojin Jafari, Gwendolyn M. Cramer, Hamid El-Hamidi, Jonathan P. Celli, Univ. of Massachusetts Boston (United States)

Invasive solid tumors are typically associated with some degree of desmoplastic reaction, marked by development of abundant stroma with collagen-rich extracellular matrix (ECM). Our lab has recently noted that phenotypic changes driven by the rheology and composition of surrounding ECM components also determine response to photodynamic therapy (PDT). Here, we look at the inverse effect, examining the direct impact of PDT on ECM composition and rheology. Notably, photochemistry subsequent to activation of PS in the presence of collagen is well known to catalyze collagen cross-linking. Using 3D models of pancreatic cancer, a disease noted for particularly abundant collagen-rich stroma, we quantify changes in ECM rigidity due to collagen photocrosslinking (PXL). Bulk rheology analysis of PDT-treated ECM shows that these effects are strongly dose dependent, with increasing ECM rigidity at low light doses, giving way to net photodestruction of ECM at higher light doses. We further examine subsequent effects on phenotype and cell motility concomitant with PDT-induced changes in ECM structure. It has been previously noted by others that subthreshold PDT doses also result in modulation of cell-ECM adhesion molecules, adding complexity to interpretation of the downstream impacts on tumor cells caused by photomodulation of the ECM itself. In view of broader recognition of the prominent role of the stroma in determining tumor progression, the results here may generate both basic insight, and therapeutic opportunities for targeting stromal interactions with PDT.

10047-5, Session 2

In vivo wide-field multispectral dosimeter for use in ALA-PpIX based photodynamic therapy

Ethan LaRochelle, Scott C. Davis, Ana Luiza Ribeiro de Souza, Stephen C. Kanick, Thayer School of Engineering at Dartmouth (United States); Michael S. Chapman M.D., Dartmouth Hitchcock Medical Ctr. (United States); Tayyaba Hasan, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

Photodynamic therapy (PDT) for Actinic Kertoses (AK) using aminoluvelinic acid (ALA) is an FDA-approved treatment, which is generally effective, yet response rates vary. The origin of the variability is not well characterized, but may be related to inter-patient variability in the production of protoporphyrin IX (PpIX). While fiber-based point probe systems provide a method for measuring PpIX production, these measurements have demonstrated large spatial and inter-operator variability. Thus, in an effort to improve patient-specific dosimetry and treatment it is important to develop a robust system that accounts for spatial variability and reduces the chance of operator errors. To address this need, a wide-field multispectral imaging system was developed that is capable of quantifying maps of PpIX in both liquid phantoms and in vivo experiments, focusing on high sensitivity light signals. The system uses both red and blue excitation to elicit a fluorescent response at varying skin depths. A ten-position filter wheel with bandpass filters ranging from 635nm to 710nm are used to capture images along the emission band. A linear least-square spectral fitting algorithm provides the ability to decouple background autofluorescence from PpIX fluorescence, which has improved the system sensitivity by an order of magnitude, detecting nanomolar PpIX concentrations in liquid phantoms in the presence of 2% whole blood and 2% intralipid. Using Monte Carlo simulations, we are able to compare the distribution of the origin of fluorescence to experimental results. These simulations are the basis for models to predict concentration and depth of PpIX production for in vivo data.

10047-6, Session 2

Quantification of localized total tissue pressure and extracellular matrix components as related to vascular patency and verteporfin uptake within pancreatic cancer

Michael D. Nieskoski, Kayla Marra, Jason R. Gunn, Thayer School of Engineering at Dartmouth (United States); Marvin M. Doyley, Univ. of Rochester (United States); Kimberly S. Samkoe, Thayer School of Engineering at Dartmouth (United States); Stephen P. Pereira, Univ. College London (United Kingdom); B. Stuart Trembly, Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

Pancreatic tumors are characterized by large interstitial hypertension from enhanced deposition of extracellular matrix components, resulting in widespread vascular collapse and reduced molecular uptake of systemically delivered therapies. Although the origins of hypoperfusion is debated amongst researchers, spatial distribution of collagen density and hyaluronic acid content have shown to be a key metric in understanding the lack of efficacy for both acute and chronic therapies in these tumors. In this study, the AsPC-1 tumor model was used both subcutaneously and orthotopically to study the measurable factors which are related to this. A conventional piezoelectric pressure catheter was used to measure total tissue pressure (TTP), defined as a combination of solid stress (SS) and interstitial fluid pressure (IFP), TTP = SS + IFP, in multiple locations within the tumor interstitium. Matrix components such as collagen and hyaluronic acid were scored using masson's trichrome stain and hyaluronic acid binding protein (HABP), respectively, and co-registered with values of TTP. The results show that these key measurements are related to the spatial distribution of verteporfin in the same tumors. Photodynamic treatment with verteporfin is known to ablate large regions of tumor tissue and also allow better permeability for chemotherapies. The study of spatial distribution of verteporfin in relation to stromal content and TTP will help us better control these types of combination therapies.

10047-8, Session 3

A summary of light dose distribution using an IR navigation system for Photofrin-mediated Pleural PDT *(Invited Paper)*

Timothy C. Zhu, Michele M. Kim, Yi-Hong Ong, Rozhin Penjweini, Andreea Dimofte, Andreea Dimofte, Jarod C. Finlay, Carmen E. Rodriguez, Keith A. Cengel, The Univ. of Pennsylvania Health System (United States)

An IR navigation system is developed to guide the light dose delivery during clinical PDT. This study summarizes the accuracy and the light dose delivery accuracy during Photofrin-mediated pleural PDT.

10047-9, Session 3

Oxygen measurements to improve singlet oxygen explicit dosimetry

Michele M. Kim, Rozhin Penjweini, Yi Hong Ong, Jarod C. Finlay, Timothy C. Zhu, Univ. of Pennsylvania (United States)

Photodynamic therapy (PDT) involves interactions between the three main components of light fluence, photosensitizer concentration, and oxygenation. Currently, singlet oxygen explicit dosimetry (SOED) has focused on the first two of these components. The macroscopic model to calculate reacted singlet oxygen has previously involved a fixed initial ground state oxygen concentration. A phosphorescence-based oxygen probe was used to measure ground state oxygen concentration throughout treatments for mice bearing radioactively induced fibroscarcoma tumors. Photofrin-, HPPH-, and BPD-mediated PDT was performed on mice. Model-calculated oxygen and measured oxygen was compared to evaluate the macroscopic model as well as the photochemical parameters involved. Oxygen measurements at various depths were compared to calculated values. Furthermore, we explored the use of noninvasive diffuse correlation spectroscopy (DCS) to measure tumor blood flow changes in response to PDT to improve the model calculation of reacted singlet oxygen. Mice were monitored after treatment to see the effect of oxygenation and blood flow on long-term recurrence-free survival as well as the efficacy of using reacted singlet oxygen as a predictive measure of outcome. Measurement of oxygenation and blood flow during treatment helps to improve SOED as well as confirm the photochemical parameters involved in the macroscopic model. Use of DCS in predicting oxygenation changes was also investigated.

10047-10, Session 3

The exploitation of inflammation in photodynamic therapy of pleural cancer

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The onset of inflammation is a well-known physiology in tumors treated with photodynamic therapy (PDT). After PDT, the release of danger signals causes an influx of neutrophils, activation of dendritic cells, and an eventual initiation of the adaptive immune response. However, inflammation also lies at a crucial fulcrum for treatment outcome, as it can stimulate the expression of resistance factors. Therefore, effective treatment with PDT requires an understanding of the holistic contribution of inflammation. Within, we outline two means of studying tumor inflammation in the setting of PDT. Experiments are conducted in murine models of mesothelioma, including those that incorporate surgery prior to PDT or pleural propagation of the disease. First, we use a chemiluminescent agent, luminol, to detect the influx of neutrophils by in vivo molecular imaging. This longitudinal approach allows for the repeated non-invasive monitoring of PDT-induced neutrophil influx. Data clearly identify protocol-specific differences in tumor-associated neutrophil activity. Second, we describe the application of cone-beam CT to detect the fibrosis associated with murine orthotropic mesothelioma models. This approach incorporates novel methods in image segmentation to accurately identify diffuse disease in the thoracic cavity. These studies lay the foundation for future research to correlate long-term response with local PDT-induced inflammation. Such methods in monitoring of inflammation or tumor burden will enable characterization of the consequences of combinatorial therapy (e.g., intraoperative PDT). Resulting data will guide the selection of pharmacological agents or molecular imaging techniques that respectively exploit inflammation for therapeutic or monitoring purposes.

10047-11, Session 3

Monitoring and assessment of tumor hemodynamic responses during pleural photodynamic therapy

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Intrapleural photodynamic therapy (PDT) has been used in combination with lung sparing surgery to treat patients with malignant pleural mesothelioma. The light, photosensitizers and oxygen are the three most important factors required by PDT to damage the tumor vasculature and stimulate the body's anti-tumor immune response. Tissue oxygen concentration is influenced by the blood flow, blood oxygenation and oxygen metabolism. Although tumor hemodynamic is one of the most important parameters for treatment prognosis and optimization, it is not routinely monitored during PDT due to the limitation in reliable technology. In this study, we demonstrated a hybrid hemodynamic monitoring system consisting of a frequency domain diffuse optical spectroscopy and a diffuse correlation spectroscopy to measure tissue oxygenated hemoglobin (HbO2), deoxygenated hemoglobin (Hb), total hemoglobin concentration (THC), tissue oxygen saturation (StO2) and relative blood flow (rBF) concurrently during Photofrin-mediated PDT. A contact probe with three source and detectors separations (0.4, 0.7 and 1.0 cm) permitting light to penetrate to depths of 0.2 to 0.5 mm, was placed on the posterior mediastinum in the lung cavity of the patients after the surgical resection of the pleural mesothelioma and the hemodynamic quantities were measured pre and during the PDT treatment. The pre-PDT measurements were used as the baseline values to study the relative changes in the hemodynamic quantities in response to PDT. Measurements and functional assessments of tumor hemodynamic responses during PDT could provide useful insight for early prediction of long-term treatment outcomes, thus enabling clinicians to optimize and personalize treatment.

10047-13, Session 3

Phototoxic effects of free phthalocyanine and phthalocyanine conjugated to gold nanoparticles for targeted photodynamic therapy of melanoma cancer

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Photodynamic therapy (PDT) has emerged as an effective treatment modality for various malignant neoplasia and diseases. In PDT, the photochemical interaction of photosensitizer (PS), light and molecular oxygen produces singlet oxygen which can lead to tumour cell apoptosis, necrosis or autophagy. The success of PDT is limited by the hydrophobic characteristic of the PS which hinders treatment administration and efficiency. To circumvent this limitation, PS can be incorporated in nanostructured drug delivery systems such as gold nanoparticles (AuNPs). In this study, we investigated the effectiveness of free zinc monocarboxyphenoxy phthalocyanine (ZnMCPPc) and ZnMCPPc conjugated to AuNPs. Commercially purchased melanoma cancer cells cultured as cell monolayers were used in this study. Changes in cellular response were evaluated using cellular morphology, viability, proliferation, cytotoxicity and mitochondrial membrane potential (MMP). Untreated cells showed no changes in cellular morphology, proliferation, cytotoxicity and MMP.

However, photoactivated free ZnMCPPc and ZnMCPPc conjugated to AuNPs showed changes in cellular morphology and a dose dependent decrease in cellular viability and proliferation as well as an increase in cell membrane. ZnMCPPc conjugated to AuNPs showed an improved efficiency in PDT as compared to free ZnMCPPc, which might be as a result of the vehicle effect of AuNPs. Both PSs used in this study were effective in inducing cell death with ZnMCPPc conjugated to AuNPs showing great potential as an effective PS for PDT.

10047-12, Session 4

X-ray induced photodynamic therapy for cancer treatment *(Invited Paper)*

Jin Xie, The Univ. of Georgia (United States)

Photodynamic therapy (PDT) has shown great promise in cancer treatment but its applications are restrained by the shallow penetration of light (< 1 cm). To address the issue, we have developed a technology called X-ray induced PDT, or X-PDT. As the name tells, X-PDT uses X-ray, which affords great tissue penetration, to trigger a PDT process. The key component of the X-PDT technology is an integrated nanosystem called X-ray nanosensitizer (abbreviated as nanosensitizer), which consists of: 1) a nanoparticle scintillator that converts X-ray photos to visible photons; 2) photosensitizers whose excitation matches the emission of the scintillator nanoparticle; and 3) an appropriate coating that encapsulates the two. Upon X-ray irradiation, the nanoscintillator works as a transducer, producing X-ray excited optical luminescence (XEOL); the visible photons, in turn, activate the photosensitizers, producing reactive oxygen species (ROS), most importantly singlet oxygen (1O2). We have assessed the feasibility of the approach with MC540-SrAl2O4:Eu@SiO2 particles, and more recently, with NC-LiGa5O8:Cr@mSiO2 particles. We show that X-PDT can be activated by X-ray from under thick tissues (>4.5 cm) to efficiently kill cancer cells. More excitingly, our recent research finds that X-PDT is more than a simple derivative of PDT; rather, it is essentially a unique combination of PDT and radiation therapy (RT). The two modalities target different cellular components; the combination overwhelms cellular repairs, leading to synergistic therapy outcomes. These unique features underscore the great potential of X-PDT in clinical translation as a novel therapy methodology.

10047-14, Session 4

Porphyrin- and chlorin-based nanoscale metal-organic frameworks for photodynamic therapy of cancers *(Invited Paper)*

Wenbin Lin, The Univ. of Chicago (United States)

Photodynamic therapy (PDT) is an effective anticancer procedure that relies on tumor localization of a photosensitizer followed by light activation to generate cytotoxic reactive oxygen species. We recently reported the rational design of a Hf-porphyrin nanoscale metal-organic framework, DBP-UiO, as an exceptionally effective photosensitizer for PDT of resistant head and neck cancer. DBP-UiO efficiently generates singlet oxygen owing to site isolation of porphyrin ligands, enhanced intersystem crossing by heavy Hf centers, and facile singlet oxygen diffusion through porous DBP-UiO nanoplates. Consequently, DBP-UiO displayed greatly enhanced PDT efficacy both in vitro and in vivo, leading to complete tumor eradication in half of the mice receiving a single DBP-UiO dose and a single light exposure.

The photophysical properties of DBP-UiO are however not optimum with the lowest energy absorption at 634 nm and a relatively small extinction coefficient of 2200 M-1·cm-1. We recently designed a chlorin-based NMOF, DBC-UiO, with much improved photophysical properties and PDT efficacy in two colon cancer mouse models. Reduction of the DBP ligands in DBP-UiO to the DBC ligands in DBC-UiO led to a 13 nm red-shift and an 11-fold extinction coefficient increase of the lowest energy Q-band. While inheriting the crystallinity, stability, porosity, and nanoplate morphology of DBP-UiO, DBC-UiO sensitizes more efficient singlet oxygen generation and exhibits much enhanced photodynamic therapy (PDT) efficacy on two colon cancer mouse models as a result of its improved photophysical properties. Both apoptosis and immunogenic cell death contributed to cancer cell-killing in DBC-UiO induced PDT. Our work has thus demonstrated that NMOFs represent a new class of highly potent PDT agents and hold great promise in treating resistant cancers in the clinic.

10047-15, Session 4

A compact diode laser based all-fiber delivery system for PDT+PTT with integrated temperature sensing capabilities

Riccardo Gassino, Politecnico di Torino (Italy); Ida Kokalari, Univ. degli Studi di Torino (Italy); Alberto Vallan, Politecnico di Torino (Italy); Ivana Fenoglio, Univ. degli Studi di Torino (Italy); Guido Perrone, Politecnico di Torino (Italy)

The combination of photo-dynamic (PDT) and photo-thermal therapies (PTT) is emerging as a new treatment with huge potential outcomes in the cure of tumors. The approach relays on nanoparticles that produce an increase of temperature and simultaneously generate reactive species such as superoxide and singlet oxygen free radicals when excited through laser beams, typically in the visible range. In this paper we present a laser diode based system devised for irradiation of carbon graphenic nanoparticles, which have shown photodynamic and photo-thermal behavior when exposed to near-IR wavelengths at the upper limit of the first biological window. Indeed, the particular properties of the considered nanoparticles allows using a 9XX nm high power (~ 10W) diode source, a choice that introduces several additional advantages, such as large illuminated area and short treatment duration (due to the high power), higher penetration depth (possible treatment of deeper laying malignancies), favorable cost per emitted watt (due to their large diffusion in pump systems for high power lasers), and reduced footprint (system compactness). The induced temperature increase, however, is not easy to predict a priori from laser power and exposure time and traditional temperature sensors cannot be used since the introduce artifacts due to the interaction with the laser light. Therefore, to overcome these limitations, we propose a probe that integrates in the delivery fiber some Bragg gratings used as temperature sensors. Experimental validations through EPR and temperature measurements on a series of carbon nanoparticle samples are provided to assess the effectiveness of the developed system.

10047-16, Session 5

Modulating inflammation-mediated activation of growth factor/survival pathways: a novel mechanism to improve outcomes in patients undergoing intraoperative adjuvant photodynamic therapy? *(Invited Paper)*

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Adjuvant photodynamic therapy (PDT) may increase local control in patients undergoing surgical resection of serosal (pleural or peritoneal) malignancies, such as Non-small cell lung cancer (NSCLC), malignant pleural mesothelioma (MPM) or ovarian cancer (OvCa). In this study, we found that patients undergoing surgery/PDT with median time to local recurrence of <12 months demonstrated elevated EGFR/STAT3 expression levels and

increased plasma IL-6 after tumor resection as compared to patients with a median time to local recurrence of > 12 months. In vivo studies in Balbc mice undergoing incomplete surgical resection of Ab12 MPM flank tumors demonstrated activation of STAT3 and EGFR signaling as well as increased plasma IL-6. Moreover, this activation was associated with decreased efficacy of postoperative PDT as compared to mice bearing equivalent sized tumors undergoing PDT without surgery and the effects of surgery on PDT were abolished by pretreating animals with the Cox-2 inhibitor celecoxib. Finally, using 3D cell culture system in either MPM or OvCa cells, we found that activation of EGFR/STAT3/Cox-2 signaling pathways significantly decreased PDT mediated cellular cytotoxicity and inhibition of these pathways enhanced PDT efficacy. Both EGFR and STAT3 are activated in the wound healing/inflammatory response to surgical injury, and EGFR/STAT3 activation leads to increased cox-2 expression. Taken together, these observations suggests that surgically mediated activation of inflammatory signaling pathways results in STAT-3 dependent crosstalk to growth/survival pathways that impairs the potential efficacy of intraoperative/post-operative PDT. Conversely, inhibition of this response to surgery might enhance clinical outcomes w in patients with serosal malignancies.

10047-17, Session 5

Metronomic photodynamic therapy with continuous blue light exposure: a pain-free and effective treatment for human actinic keratoses *(Invited Paper)*

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Prickly, stinging pain during illumination is an undesirable, sometimes unbearable side effect of aminolevulinic acid (ALA)-based photodynamic therapy (PDT) of actinic keratoses (AK). In studies of "daylight PDT", patients report little pain, suggesting that low-level production of PpIX coupled with continuous photoactivation might allow killing of precancerous cells while limiting PpIX diffusion into nearby nerves. To test this hypothesis, we conducted a clinical trial of metronomic ALA-PDT. Twenty-four patients with AK of the face and scalp were enrolled in a bilaterally controlled, intra-patient study. AK lesions were counted, and the face was randomly assigned (subdivided) into two halves A and B. An ALA solution (20%) was applied to both halves. Side B was covered with a cloth, and Side A was immediately exposed to 405 nm blue light for 30, 45, or 60 min. At 1 hour post-ALA application, Side B was uncovered and exposed for 1000 sec (conventional regimen) while Side A was shielded. Patients reported pain on a 0-10 visual-analog scale. For every patient, pain scores were markedly lower on Side A than Side B. At the Day 4 follow-up visit, intensity of erythema was generally indistinguishable between the two sides. At the final Month 3 visit, a few patients in the 30' group had metronomic treatment responses inferior to the conventional regimen. However, for patients in the 45' and 60' groups, the reduction in AK counts was equivalent on both sides. In summary, our new PDT regimen is pain-free and effective, offering important benefits for patient care.

10047-18, Session 5

Clinical applications of PDT in lung cancer *(Invited Paper)*

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External-beam photodynamic therapy (PDT) is an FDA approved modality for non-small cell lung cancer (NSCLC) and is highly efficacious for early-stage and superficial endobronchial lesions less than 1-cm in thickness. Early studies have shown that thicker lesions may be amenable to interstitial PDT. Intraoperative PDT has shown promise in the treatment of NSCLC with pleural spread. In addition, PDT holds a unique place in the treatment of squamous cell carcinoma in situ (CIS) which may be found in the airway with endobronchial screening in high risk patients. However few studies have examined either the patient characteristics or tumor microenvironment components that affect the response to PDT. The National Lung and Esophageal Cancer PDT Registry (NLEPR) was created to explore how various patient and disease characteristics contribute to efficacy of PDT in the treatment of lung disease. Roswell Park Cancer Institute (RPCI) data will be presented regarding the PDT treatment of CIS. RPCI is partnering with Pinnacle / Concordia Healthcare to create a Center of Excellence (COE) to expand the NLEPR internationally, and to expand it beyond disease sites of lung and esophageal cancer. Initial results analyses of NLEPR data will be presented

10047-19, Session 5

Minimally toxic approach for treatment of cutaneous breast cancer metastases: capecitabine-enhanced photodynamic therapy

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Cutaneous metastasis (CM) occurs in 20% of patients with breast carcinoma (BCA), and is extremely difficult to treat. These CM are relatively resistant to chemotherapy, generally responding to ionizing radiation (IR). Multiple rounds of IR, however, lead to debilitating fibrosis and radiation dermatitis. An alternative to IR is badly needed for better management of BCA/CM. In our laboratory, we have developed differentiation-enhanced combination PDT (cPDT), a concept in which a pro-differentiating agent (methotrexate; vitamin D; or 5-fluorouracil, 5FU) is used as a neoadjuvant prior to PDT. After using these neoadjuvants, levels of protoporphyrin IX (PpIX) were elevated in animal tumor models of skin, prostate, and BCA, leading to better PDT efficacy. However, all the agents have toxicity issues. Here, we use a nontoxic 5FU precursor called Capecitabine (CPBN) for cPDT. CBPN is a standard chemotherapeutics for metastatic BCA, and is metabolized to 5FU specifically within tumor tissue. Murine (4T1) and human (MCF-7) BCA cell lines were injected into breast fat pads of nude mice. After tumor nodules appeared, CPBN (400-600 mg/kg/day) was administered by oral gavage for five days followed by intraperitoneal ALA administration on day 6. Mice were sacrificed and tumors harvested. CPBN pretreatment led to a 4-fold elevation of PpIX levels in tumors, relative to vehicle control. Not only did PpIX levels increased, but also PpIX distribution became more homogeneous after CPBN pretreatment. In summary, the use of non-toxic CPBN as a neoadjuvant prior to PDT is a combination approach with significant potential for translation into the clinic.

10047-20, Session 6

Optimization of light delivery, dosimetry, and ergonomics of intraoral PDT in a low-cost, battery-operated LED-based system for global health applications

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There remains unmet clinical need for effective, low-cost therapeutics for treatment of oral cancer in regions afflicted with disproportionately high incidence from widespread abuse of chewing tobacco mixtures. Motivated by this need, we recently reported the development and preclinical testing of a battery-powered, cost-effective, LED-based light source for ALA-PDT treatment of oral cancer in resource-limited settings. This prototype device met key performance milestones for stability, irradiance, and depth of necrosis in murine tumor xenografts. Building on this work we address unresolved challenges in dosimetry and ergonomics of intraoral light delivery, for durations up to 30 minutes, that must be robust, easy to implement, and comfortable for both patient and clinician. We discuss the design of fiber coupled light applicators and an associated mounting stage to achieve uniform irradiance as well as ergonomic and stable positioning at target sites in the oral cavity (e.g. buccal mucosa and retro molar regions). In this application we also consider additional design constraints that apply where battery-powered operation may be required and photon budget is limited. To address optical performance considerations, a series of power and beam profile measurements have informed design considerations and been evaluated in performance tests in vitro and in vivo. We specifically consider dose response effects close to light aperture edges where irradiance may be non-uniform. While motivated by criteria for global health applications, we envision technology described here to be equally applicable for optimized intraoral light delivery in well-resourced clinical settings.

10047-22, Session 6

Real time laser speckle imaging monitoring vascular targeted photodynamic therapy

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Laser Speckle Imaging is a technique that has been developed for monitoring non-invasively in vivo blood flow dynamics and vascular structure with high spatial and temporal resolution. It can records the full-field spatio-temporal characteristics of microcirculation. It has often been used to study the blood flow in tumors after photodynamic therapy (PDT) but there are almost no reports on Real Time processing Laser Speckle Imaging (RTLSI) during PDT. Applying WST11, a water soluble bacteriochlorophyll derivative as photosensitizer, we were able to set up a system in which the laser used for PDT sensitization does not interfere with the coherent light used for the speckle imaging, nor saturates the detectors. This enables a better control of the VTP's timing and volume of illumination thereby providing a spatio-selective treatment to the tumor while imaging.

Our objective was to monitor the blood flow dynamics response to VTP with WST11 at a resolution of single blood vessels in and around the tumor and in real time. Importantly, incomplete vascular occlusion may result in cancer cell survival and tumor relapse. Further, knowing the dynamics of the blood flow during and after treatment, in the tumor and its surrounding blood vessels, is of great importance when planning the timing of administration of adjuvant therapies in combination with VTP.

Here we video imaged in real time rapid vascular occlusion throughout the entire tumor volume at the level of single arteries and draining veins monitored during and after WST11-VTP treatment. At the end of illumination (10 minutes), some blood vessels seem to recover, however, these blood vessels undergo a complete arrest within a few hours after treatment. Primary occlusion of feeding arteries and draining veins is observed in agreement with previous data published by our group.

10047-23, Session 6

Intraoperative Photodynamic Treatment for High-Grade Gliomas

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Glioblastoma Multiform (GBM) is the most common primary brain tumor. Its incidence is estimated at 5 new cases each year for 100 000 inhabitants. Despite reference treatment, including surgery, radiation oncology and chemotherapy, GBM still has a very poor prognosis (median survival below 14.5 months). Because of a systematic relapse of the tumor, the main challenge is to improve local control. In this context, PhotoDynamic Therapy (PDT) may offer a new treatment modality.

GBM recurrence mainly occurs inside the surgical cavity borders. Thus, a new light applicator was designed for delivering light during a PDT procedure on surgical cavity borders after Fluorescence Guided Resection. This device combines an inflatable balloon and a guide maintaining a light source at the center while keeping the balloon under pressure.

Several experimentations (temperature and impermeability tests, homogeneity of the light distribution and ex-vivo studies) were conducted to characterize the device. A prescription abacus was created to determine illumination time from the balloon volume in order to reach a fluence value close to 25J/cm? at 5 mm inside brain tissues.

According to our experience, cavity volumes usually observed in the neurosurgery department lead to an acceptable average lighting duration, from 20 to 40 minutes. Thus, extra-time needed for PDT remains suitable with anesthesia constraints. A pilot clinical trial is planned to start in 2017 in our institution (Ethical approval pending). In view of the encouraging results observed in preclinical or clinical, the PDT treatment can quickly strengthen the armamentarium of GBM management.

10047-27, Session 6

Chemoresistant and ECM-invading pancreatic tumor populations exhibit increased sensitivity to photodynamic therapy

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Pancreatic ductal adenocarcinoma (PDAC) is associated with pronounced stromal involvement that influences tumor growth, invasiveness, and therapeutic response. The extracellular matrix (ECM)-rich PDAC stroma has been implicated as a barrier to drug penetration, but stromal depletion strategies have been disappointing. It remains unclear how the ECM, as a biophysical regulator of PDAC phenotype, regulates sensitivity and resistance to specific therapies. In this context, we use an integrative approach to evaluate the impact of differential growth and invasive behavior in rheologically-characterized ECM materials on response to chemotherapy and photodynamic therapy (PDT). In 3D cultures transplanted into ECM conditions that promote invasive behavior, we find that response to PDT is enhanced in highly motile ECM-infiltrating populations, which also become chemoresistant. We further generate chemoresistant PDAC sublines with enhanced invasive potential to compare differential response in identical ECM conditions, monitored by particle tracking microrheology measurements of dynamic matrix remodeling. In both scenarios, ECM infiltrating populations exhibit increased sensitivity to PDT. However, while

ECM-invading, chemoresistant cells have a mesenchymal phenotype, induction of epithelial-mesenchymal transition (EMT) in monolayers without ECM present is not sufficient to enhance PDT sensitivity, yet imparts chemoresistance as expected. Together, these results reveal that ECM-mediated invasive PDAC populations remain responsive to PDT in conditions that induce chemoresistance.

10047-24, Session 7

A comprehensive computational approach for simulating interstitial photodynamic therapy

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Interstitial Photodynamic Therapy (I-PDT) is a promising treatment modality for locally advanced tumors that failed to respond to standard therapy. The response to I-PDT depends on light dose rate, dose, photosensitizer concentration, oxygen pressure, blood flow and volume and host immune response. Presently, there is no universal model that can be used to predict clinical outcomes following I-PDT. In this paper we present a computational approach that aims to take into account all the above variables to model the I-PDT induced photoreaction, with the exception of immune response.

We employed our finite element model approach to compute the light propagation and absorption in conjunction with newly developed computer software (Simphotek) to model I-PDT induced photoreaction. As a proof of principal, the simulations were conducted in three-dimensional geometries, representing target tumor, surrounding anatomical features such as bone, tissue, and major blood vessels, which were constructed from computed tomography (CT) scans of patients with locally advanced head and neck or lung tumors.

The simulations suggest that we can present maps of singlet oxygen and light dose and dose rate distribution that could be compared to CT scans that are routinely used to diagnose tumor response. This study was support in part by NCI/NIH award 1RO1CA193610 to GS.

10047-25, Session 7

Multimodal OCT for assessment of vasculature-targeted PDT success

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In the study we performed M-mode-like OCT (MML OCT) monitoring of microvasculature reaction in early period post PDT. Investigation was carried out on ear model CT-26. The PDT was performed with Photoditazin (N-dimethylglucamine salt of Chlorine E6 Veta-Grand, Russia) The tumors were irradiated by 662 nm laser in dose 100 J/cm2 and power density 100 mW/cm2. MML OCT images of blood vessels were obtained before PDT, immediately after PDT and 24 hours post PDT. Accumulation of the photosensitizer in the tumor and its photobleaching were detected by fluorescence imaging in vivo At 7 days after PDT, animals were sacrificed for histopathology examination of tumors. Different microvasculature reaction after the same regime of PDT was observed. No correlation was revealed between photobleaching and tumor growth inhibition. We showed that the main mechanism of vasculature PDT was complete thrombosis of blood vessels and hemorrhage, which can be visualized by MML OCT. Relationship between microvasculature damage in early period post PDT (during 24 hours) and tumor regress/regrowth was demonstrated by MML OCT and confirmed by histology. Responder tumors, characterized by no visible blood vessels on MML OCT images during 0-24h after PDT. The advantages of MML OCT such as a simple and fast process of images obtaining, no labeling assessment and cheap approach make this method perspective in clinical routine monitoring antitumor therapies.

10047-26, Session 7

Overview of computational simulations for PDT treatments based on optimal choice of singlet oxygen dose

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Effective photodynamic therapy treatment planning and treatment monitoring requires computer simulations of both light transport in tissue and photosensitizer (PS) photophysics to estimate singlet oxygen. Simply using light dose (fluence) or PDT dose [(fluence)*(PS concentration)] as fixed values for treating all patients does not account for differences in the amount of singlet oxygen formed when fluence rates change or patient tissue parameters change. We will focus on singlet oxygen dose which is calculated by solving the photokinetics rate equations and which determines the effectiveness of the subsequent reactions of singlet oxygen with the cancer target and PS. We will show that solutions based on the complete system of the rate equations and on the reduced system (approximate solution) are equivalent for both regimes: for short times (< 2 s) as well as for longer times (> 2s). We discuss some of the issues that can make dosimetry, based on singlet oxygen dose, inaccurate. We will confirm sensitivity of singlet oxygen dose to the patient-related parameters (absorption, scattering and oxygen intake rate), real-time monitoring of which can improve outcomes compared to just using fixed values prescribed during pre-treatment planning. We will show that fluence-only based dosimetry cannot be optimal and has its limitations.

10047-28, Session 7

Simple and optimum background-free estimation method of PPIX fluorescence for 5-ALA-based fluorescence diagnosis of malignant lesions

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Accurate and rapid evaluation of lymph node metastasis is required in tumor staging and the decision of treatment strategy. General intraoperative pathological evaluation, however, takes at least a few tens of minutes or longer for metastasis diagnosis. 5-aminolevulinic acid (5-ALA)-based fluorescence diagnosis is a solution for accurate and ultrarapid diagnosis of malignant lesions. 5-ALA-based diagnosis evaluates fluorescence intensity of a fluorescent metabolite of 5-ALA, protoporphyrin IX (PPIX); however, the fluorescence of PPIX is often affected by autofluorescence of tissue chromophores, such as collagen and flavins. To enhance the accuracy of the diagnosis of malignant lesions based on the PPIX fluorescence, elimination of the autofluroescence is required. In this study, we proposed and experimentally demonstrated background-free PPIX fluorescence estimation method by simplified and optimized multispectral imaging. To realize background-free PPIX fluorescence estimation, we computationally optimized observation wavelength regions in terms of minimizing prediction error of PPIX fluorescence intensity in the presence of typical chromophores, collagen and flavins. We verified the fundamental detection capability of our method by using known-chemical mixtures. Furthermore, we applied our method to lymph node metastasis, and successfully realized background-free histopathological evaluation of metastatic lesions of lymph node metastasis. Our results confirmed the potential of the background-free estimation method of PPIX fluorescence for 5-ALA-based fluorescence diagnosis of malignant lesions, and we expect this method to be beneficial for intraoperative and rapid cancer diagnosis.

10047-29, Session 8

Threshold distributions in photodynamic therapy of normal, tumor and resistant cell lines

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Experimental Photodynamic Therapy (PDT), either in vivo or in vitro, is normally carried out under distinct conditions making it difficult to compare results in order to propose the best combination for optimized outcomes. In this work, a threshold distribution model was used to investigate the PDT response in vitro. It is known that different types of cells present distinguished resistance to treatment, which can be due to several factors. The threshold distribution obtained from the differentiation of the dose-response curves, is under discussion by several authors. The main parameters of the distribution are related with the most frequent threshold in the population, given by the dose of the peak, and its variability is represented by the the distribution width. To evaluate how PDT response differs, we used normal and tumor cell lines from liver (HepaRG, HepG2, respectively) and breast tissues (MCF-7 and HMEC). We also performed an induction protocol of tumor resistance to assess the variations in the threshold distributions of the derived cells. Results show that the normal cell lines generally present a more homogenous response since the threshold distributions are more symmetric and narrower than the ones from the tumor cell lines. We also observed that MCF-7 is more resistant to PDT than HepaRG and HepG2. Experiments to investigate the causes for the different responses, such as photosensitizer uptake and reactive oxygen species (ROS) production, were performed. The findings are promising and encourage the further investigation ofvariability in PDT responses using the threshold distribution model.

10047-30, Session 8

Novel trace norm regularization method for fluorescence molecular tomography reconstruction

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Fluorescence molecular tomography (FMT) is developing rapidly in the field of molecular imaging. FMT has been used in surgical navigation for tumor resection and has many potential applications at the physiological, metabolic, and molecular levels in tissues. Due to the ill-posed nature of the problem, which is caused by the high degree of absorption and scattering of light through tissue, many regularized methods are generally adopted. In this paper, we propose a region reconstruction method for FMT in which the trace norm regularization. The trace norm penalty was defined as the sum of the singular values of the matrix. The proposed method adopts a priori information which is the structured sparsity of the fluorescent regions for FMT reconstruction. In order to improve the solution efficiency, the accelerated proximal gradient algorithms was used to accelerate the computation. The numerical phantom experiment was conducted to evaluate the performance of the proposed trace norm regularization method. Meanwhile, in order to quantitatively evaluate the performance of the reconstruction results, the position error (PE) and contrast-to-noise ratio (CNR) are introduced in this paper. The simulation study shows that the proposed method achieves accurate and is able to reconstruct image effectively.

10047-31, Session 8

Real-time monitoring of tumor micro-environment during photoimmunotherapy in vivo

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Photo-immunotherapy (PIT) is an emerging low-side-effect cancer therapy based on monoclonal antibody (mAb) conjugated with a near-infrared (NIR) phthalocyanine dye IRDye700DX (IR700 is not only fluorescent which can be used as an imaging agent, but also phototoxic) that induces rapid cell death after exposure to NIR light. PIT induces highly-selective cancer cell death while leaving most of tumor blood vessels unharmed, leading to an effect termed super-enhanced permeability and retention (SUPR), which significantly improve the effectiveness of anti-cancer drug. Currently, the therapeutic effects of PIT were monitored using IR700 fluorescent signal based on macroscopic fluorescence reflectance imager, which lacks the resolution and depth information to reveal the intra-tumor heterogeneity of mAb-IR700 distribution. We developed a minimally-invasive two-channel fluorescence fiber imaging system by combining the traditional fluorescence imaging microscope with two imaging fiber bundles (~0.85 mm) to monitor mAb-IR700 distribution and therapeutic effects during PIT at different intra-tumor locations (e.g. tumor periphery vs. tumor rim) in situ and in real time simutaneously, thereby enabling evaluation of the therapeutic effects in vivo and optimization of treatment regimens accordingly. Experiments were carried out on ten mice. The average fluorescence intensity recovery after PIT in tumor rim is 91.50% while 100.63% in tumor periphery. Significantly

higher fluorescence redistribution (P=0.0371) in tumor periphery than tumor rim after PIT treatment were observed. In order to verify the results, two-photon microscopy combining with micro-prism was also used to record the mAb-IR700 distribution at different depth locations of the tumor during PIT.

10047-32, Session 8

Cypate-mediated thermosensitive nanoliposomes for nir imaging and photothermal triggered drug release

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The more and more excessive treatments and the resulting serious pain the patients get in the whole period of treatment call for precise diagnosis and effective as well as comprehensive treatments. In this work, we developed a kind of multi-functional photo-thermal sensitive liposomes (PTSL), by doping cypate, with the abilities of near infrared (NIR) imaging, photo-thermo conversion and singlet oxygen generating, into hydrophobic layer of the synthesized thermosensitive liposomes (TSL). Based on the PTSL, a theranostic nanocomposite, DOX@PTSL was established with doxorubicin (DOX) entrapped into the hydrophilic layer of PTSL. The physico-chemical properties were characterized by laser particle size analyzer, transmission electron microscope (TEM), UV-Visable spectrophotometer, infrared thermal imager and differential scanning calorimetry (DSC). For cell experiments, the MTT assay was performd to study the cell toxicity and the confocal laser scanning microscope (CLSM) was used to observe the locating properties and dynamics of DOX@PTSL after incubated with U87 cell lines and irradiated with NIR laser. The biodistribution, thermal images, and therapeutic effects of DOX@PTSL on tumor-bearing mice were investigated in the animal experiments. The size of liposome we synthesized is 90 to 190 nm while the phase inversion temperature falls on 43.1°C. And importantly, then drug release can be traggered by NIR laser, with almost all drugs entering the tumor cells' nucleus. In general, the DOX@PTSL was demonstrated tumor-targeted abilities, enhanced antitumor activities, minimal side effects and significant improved capability to combat with free DOX as results of the comprehensive therapeutic effectiveness of chemotherapy, thermotherapy and cypate-mediated photochemical internalization.

10047-33, Session PMon

Singlet oxygen explicit dosimetry to predict long-term local tumor control for BPD-mediated photodynamic therapy

Michele M. Kim, Rozhin Penjweini, Timothy C. Zhu, Univ. of Pennsylvania (United States)

Photodynamic therapy (PDT) is a well-established treatment modality for cancer and other malignant diseases; however, quantities such as light fluence, photosensitizer photobleaching rate, and PDT dose do not fully account for all of the dynamic interactions between the key components involved. In particular, fluence rate (?) effects are not accounted for, which has a large effect on the oxygen consumption rate. In this preclinical study, reacted singlet oxygen ([1O2]rx) was investigated as a dosimetric quantity for PDT outcome. The ability of [1O2]rx to predict the long-term local tumor control rate (LCR) for BPD-mediated PDT was examined. Mice bearing radioactively-induced fibrosarcoma (RIF) tumors were treated with different in-air fluences (250, 300, and 350 J/cm2) and in-air ? (75, 100, and150 mW/cm2) with a BPD dose of 1 mg/kg and a drug-light interval of 3 hours. Treatment was delivered with a collimated laser beam of 1 cm diameter at 690 nm. Explicit dosimetry of initial tissue oxygen concentration, tissue optical properties, and BPD concentration was used to calculate [1O2]rx. ? was calculated for the treatment volume based on Monte-Carlo simulations and measured tissue optical properties. Kaplan-Meier analyses for LCR were done for an endpoint of tumor volume ≤ 100 mm3 using four dose metrics: light fluence, photosensitizer photobleaching rate, PDT dose, and [1O2]rx. PDT dose was defined as the product of the time-integral of photosensitizer concentration and ? at a 3 mm tumor depth. Preliminary studies show that [1O2]rx better correlates with LCR and is an effective dosimetric quantity that can predict treatment outcome.

10047-34, Session PMon

3-compartment dynamic model of talaporfin sodium pharmacokinetics in silico

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We studied a 3-compartment dynamic model of talaporfin sodium pharmacokinetics in silico. Drug distribution might change after intravenous injection from plasma to interstitial space and then into the cells. We have developed a new cardiac ablation using photosensitization reaction with laser irradiation shortly after talaporfin sodium injection. We think that the major cell-killing factor in our cardiac ablation would be an oxidation by singlet oxygen produced in the interstitial space in myocardium with laser irradiation shortly after the photosensitizer administration. So that the talaporfin sodium concentration change in time in the interstitial space should be investigated. We constructed the pharmacokinetics dynamic model composed by 3-compartments, that is, plasma, interstitial space, and cell. We measured talaporfin sodium fluorescence time change in human skin by our developed fluorescence measurement system in vivo. Using the measured concentration data in plasma and skin in human, we verified the calculation accuracy of our in silico model. We compared the simulated transition tendency from interstitial space to cells in our in silico model with the reported uptake tendency using cell. We identified the transition coefficients between plasma, interstitial space, and cell compartment, and metabolization coefficient from plasma by the fitting with measured data.

10047-35, Session PMon

Diffuse reflectance images to detect absorbing inclusions in scattering media

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Biological tissues are mostly complex, non-homogeneous and optically highly scattering structures. Despite the hundreds of existent studies on the propagation of light in biological tissues, its complexity requires new studies to be conducted in order to improve the existing knowledge, which still has many gaps. The presence of heterogeneities in tissue changes the light propagation and impedes the predictability of its behavior by mathematical models. This work aimed to establish an empirical method using diffuse reflectance images acquired with simple instrumentation, based on a continuous light source at 660 nm and a monochromatic CMOS camera, to check the possibility of detecting absorbing inclusions embedded in highly scattering phantoms. The ability to detect inclusions of two different kinds of materials in different sizes and geometries, positioned at different depths were evaluated. The incident angle was also varied, as well as the distance between the source and the object, in order to evaluate the best experimental conditions. The results showed that the objects could be detected, and their shapes might be satisfactorily recovered by an algorithm developed for image processing. In some situations, even at the greatest depth used, which was 20 mm, the inclusion could be detected in diffuse reflectance processed images. Although the detection capability of geometric shapes represents an improvement over the structures of identification possibilities in turbid media, the determination of depth is still a challenge to be overcome.

10047-36, Session PMon

Singlet oxygen explicit dosimetry to predict local tumor control for HPPH-mediated photodynamic therapy

Rozhin Penjweini, Michele M. Kim, Timothy C. Zhu, Univ. of Pennsylvania (United States)

This preclinical study examines four dosimetric quantities (light fluence, photosensitizer photobleaching ratio, PDT dose, and reacted singlet oxygen ([1O2]rx)) to predict local control rate (LCR) for 2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide (HPPH)-mediated photodynamic therapy (PDT). Mice bearing radiation-induced fibrosarcoma (RIF) tumors were treated with different in-air fluences (135, 250 and 350 J/cm2) and in-air fluence rates (50, 75 and 150 mW/cm2) at 0.25 mg/kg HPPH and a drug-light interval of 24 hours using a 1 cm diameter collimated laser beam at 665 nm wavelength. A macroscopic model was used to calculate [1O2]rx based on in vivo explicit dosimetry of the initial tissue oxygenation, photosensitizer concentration, and tissue optical properties. PDT dose was defined as a temporal integral of drug concentration and fluence rate (?) at a 3 mm tumor depth. Light fluence rate was calculated throughout the treatment volume based on Monte-Carlo simulation and measured tissue optical properties. The tumor volume of each mouse was tracked for 30 days after PDT and Kaplan-Meier analyses for LCR were performed based on a tumor volume ≤100 mm3, for four dose metrics: fluence, HPPH photobleaching rate, PDT dose, and [1O2]rx. The results of this study showed that [1O2]rx is the best dosimetric quantity that can predict tumor response and correlate with LCR.

10047-37, Session PMon

Singlet oxygen explicit dosimetry to predict long-term local tumor control for Photofrin-mediated photodynamic therapy

Rozhin Penjweini, Michele M. Kim, Yi-Hong Ong, Timothy C. Zhu, Univ. of Pennsylvania (United States)

Although photodynamic therapy (PDT) is an established modality for the treatment of cancer, current dosimetric quantities do not account for the variations in PDT oxygen consumption for different fluence rates. In this study we examine the efficacy of reacted singlet oxygen concentration ([1O2]rx) to predict long-term local control rate (LCR) for Photofrin-mediated PDT. Radiation-induced fibrosarcoma (RIF) tumors in the right shoulders of female C3H mice are treated with different in-air fluences (135 and 250 J/cm2) and in-air fluence rates (50 and 75 mW/cm2) at 5 mg/kg Photofrin and a drug-light interval of 24 hours using a 1 cm diameter collimated laser beam at 630 nm wavelength. [1O2]rx is calculated by using a macroscopic model based on explicit dosimetry of Photofrin concentration, tissue optical properties, tissue oxygenation and blood flow changes during PDT. The tumor volume of each mouse is tracked for 30 days after PDT and Kaplan-Meier analyses for LCR are performed based on a tumor volume ≤100 mm3, for the four dose metrics light fluence, photosensitizer photobleaching rate, PDT dose and [1O2]rx. PDT dose is defined as a temporal integral of photosensitizer concentration and fluence rate at a 3 mm tumor depth. Fluence rate is calculated throughout the treatment volume based on Monte-Carlo simulation and measured tissue optical properties. Our preliminary studies show that [1O2]rx is the best dosimetric quantity that can predict tumor response and correlate with LCR. Moreover, [1O2]rx calculated based on the blood flow changes was in agreement with [1O2]rx calculated based on the tissue oxygenation.

10047-38, Session PMon

The relevance of light diffusion profiles for interstitial PDT using light-diffusing optical fibers

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Photodynamic therapy (PDT) is a technique used for several tumor types treatment. Light penetration on biological tissue is one limiting factor for PDT applied to large tumors. An alternative is using interstitial PDT, in which optical fibers are inserted into tumors. Cylindrical diffusers have been used in interstitial PDT. Light emission of different diffusers depend on the manufacturing process, size and optical properties of fibers, which make difficult to establish an adequate light dosimetry, since usually light profile is not designed for direct tissue-fiber contact. This study discusses the relevance of light distribution by a cylindrical diffuser into a turbid lipid emulsion solution, and how parts of a single diffuser contribute to illumination. A 2 cm-long cylindrical diffuser optical fiber was connected to a diode laser (630 nm), and the light spatial distribution was measured by scanning the solution with a collection probe. From the light field profile generated by a 1 mm-long intermediary element of a 20 mm-long cylindrical diffuser, recovery of light distribution for the entire diffuser was obtained. PDT was performed in rat healthy liver for a real treatment outcome analysis. By using computational tools, a typical necrosis profile generated by the irradiation with such a diffuser fiber was reconstructed. The results showed that it was possible predicting theoretically the shape of a necrosis profile in a healthy, homogeneous tissue with reasonable accuracy. The ability to predict the necrosis profile obtained from an interstitial illumination by optical diffusers has the potential improve light dosimetry for interstitial PDT.

10047-39, Session PMon

Target-specific porphyrin-loaded hybrid nanoparticles to improve photodynamic therapy for cancer treatment

Juan Vivero-Escoto, Daniel L. Vega, The Univ. of North Carolina at Charlotte (United States)

Photodynamic therapy (PDT) has emerged as an alternative approach to chemotherapy and radiotherapy for cancer treatment. The photosensitizer (PS) is perhaps the most critical component of PDT, and continues to be an area of intense scientific research. Traditionally, PS molecules like porphyrins have dominated the field. Nevertheless, these PS agents have several disadvantages, with low water solubility, poor light absorption, and reduced selectivity for targeted tissues being some of the main drawbacks. Polysilsesquioxane (PSilQ) nanoparticles provide an interesting platform for developing PS-loaded hybrid nanocarriers. Several advantages can be foreseen by using this platform such as carrying a large payload of PS molecules; their surface and composition can be tailored to develop multifunctional systems (e.g. target-specific); and due to their small size, nanoparticles can penetrate deep into tissues and be readily internalized by cells. In this work, porphyrin-loaded PSilQ nanoparticles with a high payload of photosensitizers were synthesized, characterized, and applied in vitro. The network of this nanomaterial is formed by porphyrin-based photosensitizers chemically connected via a redox-responsive linker. Under reducing environment such as the one found in cancer cells the nanoparticles can be degraded to efficiently release single photosensitizers in the cytoplasm. The platform was further functionalized with polyethylene glycol (PEG) and folic acid as targeting ligand to improve its biocompatibility and target specificity toward cancer cells overexpressing folate receptors. The effectiveness of

this porphyrin-based hybrid nanomaterial was successfully demonstrated in vitro using human cervical, breast, and colon cancer cells.

10047-40, Session PMon

Photodynamic Therapy in Neurosurgery: A Proof of Concept of Treatment Planning System

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GlioBlastoma Multiform (GBM) is the most common primary brain tumour. PhotoDynamic Therapy (PDT) appears as an interesting research field to improve GBM treatment. Nevertheless, PDT cannot fit into the current therapeutic modalities according to several reasons: the lack of reliable and reproducible therapy schemes (devices, light delivery system), the lack of consensus on a photosensitizer and the absence of randomized and controlled multicentre clinical trial. The main objective of this study is to bring a common support for PDT planning.

Here, we describe a proof of concept of Treatment Planning System (TPS) dedicated to interstitial PDT for GBM treatment. The TPS was developed with the integrated development environment C++ Builder XE8 and the environment ArtiMED, developed in our laboratory. This software enables stereotactic registration of DICOM images, light sources insertion and an accelerated CUDA GPU dosimetry modelling.

Although, Monte-Carlo method is more robust to describe light distribution in biological tissues, analytical model accelerated by GPU remains relevant for dose preview or fast inverse planning process. Finally, this preliminary work proposes a new tool to plan interstitial or intraoperative PDT treatment and might be included in the design of future clinical trials in order to deliver PDT straightforwardly and homogenously in investigator centres.

10047-41, Session PMon

Comparison of doses delivered in daylight versus regular light delivery in ALA-PDT in the treatment of skin cancer

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Use of ALA-PDT has been approved by FDA to treat actinic keratosis and pre–cancerous lesions. However, pain is been reported as a side–effect of ALA-PDT due to PpIX production and/or uptake by nerve cells, which lead to stimulation or damages of peripheral nerve endings during illumination by reactive singlet oxygen. A strategy to reduce pain is the use of daylight PDT immediately after ALA application, since photobleaching has been correlated to pain. Moreover, previous studies have shown that the efficacy of ALA-PDT is related to cross-linking of STAT3 and it can be used as a molecular marker for the photodynamic reaction. The aim of this study was compare the use of daylight PDT (dPDT), PDT and fractionated PDT (fPDT). Intradermal implantation of SCC25 cell line was performed on nude mice. When the tumors reached 10-20 mm3, the animals were divided in 4 different groups: control, ALA-PDT, ALA-fPDT, and ALA-dPDT. Optical measurements were acquired with the white light source and 405 nm blue laser diode prior to administration of ALA, before and after PDT. The results from dosimeter showed that PpIX was re-synthesized during 2 h dark for ALA-fPDT group. Moreover, there was no statistical difference between PpIX fluorescence after ALA-dPDT and ALA-fPDT, indicating that PpIX was consumed at the same time that it was produced. Further studies are ongoing to evaluate the effect of different light treatments using STAT3 cross-linking as an independent biological dosimeter of effectiveness of photodynamic therapy to compare regular PDT to daylight PDT.

10047-42, Session PMon

Photodynamic activity of zinc monocarboxyphenoxy phthalocyanine (ZnMCPPc) conjugated to gold silver (AuAg) nanoparticles in melanoma cancer cells

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Photodynamic therapy (PDT) is a minimally invasive therapeutic modality for the treatment of neoplastic and non-neoplastic diseases. In PDT of cancer, irradiation with light of a specific wavelength leads to activation of a photosensitizer which results in generation of reactive oxygen species (ROS) which induces cell death. Many phthalocyanine photosensitizers are hydrophobic and insoluble in water, which limits their therapeutic efficiency. Consequently, advanced delivery systems and strategies are needed to improve the effectiveness of these photosensitizers. Nanoparticles have shown promising results in increasing aqueous solubility, bioavailability, stability and delivery of photosensitizers to their target. This study investigated the photodynamic activity of zinc monocarboxyphenoxy phthalocyanine (ZnMCPPc) conjugated to gold silver (AuAg) nanoparticles in melanoma cancer cells. ZnMCPPc conjugated to AuAg nanoparticles were synthesized and characterized by using transmission electron microscopy (TEM) and UV-Vis spectroscopy. The photodynamic activity of ZnMCPPc conjugated to AuAg nanoparticles was evaluated using cellular morphology, viability, proliferation, cytotoxicity and mitochondrial membrane potential (MMP). Untreated cells showed no changes in cellular morphology, proliferation, cytotoxicity and MMP. However, photoactivated ZnMCPPc conjugated to AuAg nanoparticles showed changes in cellular morphology and a dose dependent decrease in cellular viability, proliferation and MMP as well as an increase in cell membrane damage. The ZnMCPPc conjugated to AuAg nanoparticles used in this study was highly effective in inducing cell death of melanoma cancer cells.

10047-43, Session PMon

Four-channel PDT dose dosimetry for pleural photodynamic therapy

Yi Hong Ong, Michele M. Kim, Jarod C. Finlay, Timothy C. Zhu, Univ. of Pennsylvania (United States)

PDT dose is the product of the photosensitizer concentration and the light fluence in the target tissue. For improved dosimetry during Photofrin-mediated intrapleural photodynamic therapy (PDT), a four-channel PDT

dose dosimeter was developed to measure both the light fluence and the Photofrin fluorescence signal simultaneously from four different sites in the pleural cavity during PDT. An isotropic detector with bifurcated fibers was used for each channel to ensure detected light was split equally to the photodiode and spectrometer. Light fluence varies significantly due to the movement of source during PDT treatment. The fluorescence signal is normalized by the light fluence measured at treatment wavelength. We have shown that the absolute photosensitizer concentration can be obtained by applying optical properties correction and linear spectral fitting to the measured fluorescence data. Channel-specific optical property correction factors were determined in phantom experiments. The detection limit and the OP correction factor of each channel were determined and validated using tissue-simulating phantoms with known varying concentration of Photofrin. Tissue optical properties are determined using an absorption spectroscopy probe immediately before PDT at the same sites. This novel method allows accurate real-time determination of delivered PDT dose using existing isotropic detectors, and may lead to a considerable improvement of PDT treatment quality compared to the currently employed systems. Multiple sites were measured to investigate the heterogeneity of the cavity and to provide cross-validation via relative dosimetry.

10047-44, Session PMon

Temperature mapping and thermal dose calculation in combined radiation therapy and 13.56 MHz radiofrequency hyperthermia for tumor treatment

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To evaluate the synergistic effect of radiotherapy and radiofrequency hyperthermia therapy in the treatment of lung and liver cancers, we studied the mechanism of heat absorption and transfer in the tumor using electro-thermal simulation and high-resolution temperature mapping techniques. A realistic tumor-induced mouse anatomy, which was reconstructed and segmented from computed tomography images, was used to determine the thermal distribution in tumors during radiofrequency (RF) heating at 13.56 MHz. An RF electrode was used as a heat source, and computations were performed with the aid of the multiphysics simulation platform Sim4Life. Experiments were carried out on a tumor-mimicking agar phantom and a mouse tumor model to obtain a spatiotemporal temperature map and thermal dose distribution. A high temperature increase was achieved in the tumor from both the computation and measurement, which elucidated that there was selective high-energy absorption in tumor tissue compared to the normal surrounding tissues. The study allows for effective treatment planning for combined radiation and hyperthermia therapy based on the high-resolution temperature mapping and high-precision thermal dose calculation.

10047-45, Session PMon

In vivo deep tissue photothermal treatment using a light injection device based on fiber needle array device

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Photothermal treatment methods have been widely studied for their target specificity and potential for supplementing the limitations of conventional surgical treatments.

Here, we demonstrated an in vivo photothermal treatment using a custom-built light injection device which consists of eight flexible optical fibers being connected to hypodermic syringe needles. The fiber-needle array device can effectively deliver light energy into subcutaneous tissues with minimal skin damages. An image fiber bundle is placed through the center of the light injection device for real-time monitoring of the thermal treatment. We injected nanoshells at the peritumoral sites, and then the light injection device was stuck on the tumor site by about 1 mm deep into the tissue and a near-infrared laser with a wavelength of 960 nm was irradiated for 1 minute. The intensity of the light introduced by the individual fibers was about 0.5 W/cm2 used for the treatment, which is 0.5 times lower than that required for the case of direct irradiation in the our previous study. During the photothermal treatment, the lesion was monitored by the image fiber bundle and the existence of irradiated nanoshells was confirmed. Even with the low-level light irradiation by the fiber-needle array the tumor was effectively treated and thus eliminated. The effectiveness of the light delivery by the injection device was exploited and verified by a separate measurement using a tissue phantom. This method will be useful for the effective photothermal treatment and the real-time monitoring for in vivo.

10047-46, Session PMon

Dendrimer phthalocyanine based nanoparticles for enhancement in vitro photodynamic efficacy

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A novel series of nanoparticles formed via an electrostatic interaction between the periphery of negatively charged 1-2 generation aryl benzyl ether dendrimer zinc (II) phthalocyanines and positively charged poly(L-lysin) segment of triblock copolymer, poly(L-lysin)-block-poly(ethylene glycol)-block-poly(L-lysin) , was developed for the use as an effective photosensitizers in photodynamic therapy. The dynamic light scattering, atomic force microscopy showed that two nanoparticles has a relevant size of 80-150 nm. The photophysical properties and singlet oxygen quantum yields of free dendrimer phthalocyanines and nanoparticles exhibited generation dependence. The intracellular uptake of dendrimer phthalocyanines in Hela cells was significantly elevated as they were incorporated into the micelles, but was inversely correlated with the generation of dendrimer phthalocyanines. The photocytotoxicity of dendrimer phthalocyanines incorporated into polymeric micelles was also increased. The presence of nanoparticles induced efficient cell death. Using a mitochondrial-sepcific dye rhodamine 123 (Rh123), our fluorescence microscopic result indicated that nanoparticles localized to the mitochondria.

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10048-1, Session 1

Photobiomodulation and the brain: a new paradigm *(Invited Paper)*

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Photobiomodulation (PBM) describes the use of red or near-infrared light to stimulate, heal, regenerate, and protect tissue that has either been injured, is degenerating, or else is at risk of dying. One of the organ systems of the human body that is most necessary to life, and whose optimum functioning is most worried about by humankind in general, is the brain. The brain suffers from many different disorders that can be classified into three broad groupings: traumatic events (stroke, traumatic brain injury, and global ischemia), degenerative diseases (dementia, Alzheimer's and Parkinson's), and psychiatric disorders (depression, anxiety, post traumatic stress disorder). There is some evidence that all these seemingly diverse conditions can be beneficially affected by applying light to the head. There is even the possibility that PBM could be used for cognitive enhancement in normal healthy people. In this transcranial PBM (tPBM) application, near-infrared (NIR) light is often applied to the forehead because of the better penetration (no hair, longer wavelength). Some workers have used lasers, but recently the introduction of inexpensive light emitting diode (LED) arrays has allowed the development of light emitting helmets or "brain caps". This presentation will cover the mechanisms of action of photobiomodulation to the brain, and summarize some of the key pre-clinical studies and clinical trials that have been undertaken in this area.

10048-2, Session 1

Clinical trial involving sufferers and non-sufferers of cervicogenic headache (CGH): potential mechanisms of action of photobiomodulation

Ann D. Liebert, Australasian Research Institute (Australia); Brian Bicknell, Australian Catholic Univ. (Australia)

Photobiomodulation (PBM) is an effective tool for the management of spinal pain including inflammation of facet joints. Apart from cervical and lumbar joint pain the upper cervical spine facet joint inflammation can result in the CGH (traumatic or atraumatic in origin). This condition affects children, adults and elders and is responsible for 19% of chronic headache and up to 33% of patients in pain clinics. The condition responds well to physiotherapy, facet joint injection, radiofrequency neurotomy and surgery at a rate of 75%. The other 25% being unresponsive to treatment with no identified features of unresponsiveness. In other conditions of chronic unresponsive cervical pain have responded to photobiomodulation at a level of 80% in the short and medium term.

A clinical trial was therefore conducted on a cohort of atraumatic patients from the ages of 5-93 (predominantly Neurologist referred / familial sufferers 2/3 generations vertically and laterally) who had responded to a course of PBM and physiotherapy. The CGH sufferers and their non CGH suffering relatives over these generations were then compared for features that distinguish the two groups. Fifty parameters were tested (anthropmetric, movement and neural tension tests included) and there was a noted difference in tandem stance between the groups (.04 significance with repeated measures).

As this impairment is common to benign ataxia and migrainous vertigo and in these conditions there is an ion channelopathy (especially potassium channelopathy). A postulated mechanism of action of PBM would involve modulation of ion channels and this is discussed in this presentation.

10048-3, Session 1

Near infrared phototherapy and EEG biofeedback in the treatment of cognitive and behavioral symptoms of Alzheimer's disease

Marvin H. Berman, Quietmind Foundation (United States)

Evidence from animal and human studies regarding the biological impact of near infrared light stimulation has significantly increased of late noting the disease modifying properties of photobiomodulation for improving physical and cognitive performance in subjects with a variety of neurodegenerative conditions. Concurrently we see a growing body of literature regarding the efficacy of operant conditioning of EEG amplitude and connectivity in remediating both cognitive and behavioral symptoms of both neuropsychiatric and neurodegenerative disorders including traumatic brain injury, ADHD, PTSD, and dementia. This presentation seeks to outline a treatment model combining these two treatment methods to stop the progression of neurodegeneration using pulsed (10hz), brief (5-20minutes) repeated (1-2x/daily) transcranial and intranasal photobiomodulation with 810nm and 1068nm near infrared phototherapy and operant conditioning of EEG amplitude and coherence. Our initial study on treating dementia with EEG biofeedback (N=37) showed neuroplasticity's potential for modifying cognitive and behavioral symptoms using the evidence from decades of neurological research that never felt the warm touch of a translational researcher's hand. The near infrared interventional studies clarified the order of treatment, i.e., tissue health and renewal were achieved, followed by neural connectivity enhancement. Significant improvements in both immediate and delayed recall and praxis memory as well as executive functioning and behavioral regulation were obtained with each intervention. The inferred synergistic impact of properly combining these approaches is what informs our current clinical applications and future research efforts examining the value of combined treatments for all dementias, parkinson's disease and age-related dry macular degeneration.

10048-4, Session 1

Nano pulse laser therapy (NPLT) of traumatic brain injury

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Traumatic brain injury (TBI) is a major cause of death and disability in the US. We designed and built a novel, medical grade, Nano Pulse Laser Therapy (NPLT) system for transcranial delivery of NIR light and ultrasound waves to the brain. It generates nanosecond pulses (up to 15 mJ) in the range 680-1064 nm that produce low-level, MHz optoacoustic (ultrasound) waves that propagate deeper into the brain. Here we tested the efficacy of NPLT in two rodent models of TBI, a blast injury and a fluid percussion injury model. Cognitive function was assessed using a working memory paradigm of the Morris water maze test. The expression of the neuronal growth factor BDNF was measured in laser-captured cortical and hippocampal neurons. Microglia activation and neuronal injury was assessed in brain sections by immunofluorescence using specific antibodies against CD68 and activated caspase-3 respectively. The effect of NPLT on hippocampal neurogenesis was assessed using BrdU incorporation. Our results show that a 5-minute transcranial application of NPLT significantly reduces brain damage and neuroinflammation, increases neurogenesis, and preserves vestibulomotor and cognitive functions in rodent models of TBI. The data suggest that our proprietary system has a significant value for TBI therapy. These studies

were completed as part of an interdisciplinary research team funded by The Moody Project for Translational Traumatic Brain Injury Research.

10048-5, Session 1

Diffuse correlation spectroscopy (DCS) study of blood flow changes during low level laser therapy (LLLT)

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Photobiomodulation with low-power, high-fluence light in the near-infrared range (600-1100nm), also known as Low Level Laser Therapy (LLLT), has been used for reducing pain, promoting healing of wounds, and so on. Understanding its physiological and hemodynamic effects is essential for treatment optimization and evaluation. In this study, we used diffuse correlation spectroscopy (DCS) to investigate the changes of regional blood flow induced by a single session of LLLT. DCS is an emerging optical modality to probe microvascular blood flow by utilizing the temporal fluctuations of near-infrared light. We have developed a software-based autocorrelator system with the benefits such as flexibility in raw data processing, portability and low cost. LLLT was administered at the human forearm with a 1064-nm, continuous-wave laser. The emitting power was 3.4 W in an area of 13.6 cm2, corresponding to 0.25W/cm2 irradiance. The emitting duration was 10 minutes. Eight healthy adults of any ethnic background, in an age range of 18-40 years old were included. The results indicate that LLLT causes reliable changes in regional blood flow. However, it remains unclear whether these changes are physiological or attributed to the heating effect of the laser.

10048-6, Session 2

Extraorally delivered photobiomodulation therapy for prevention of oropharyngeal mucositis in pediatric patients undergoing hematopoietic cell transplantation *(Invited Paper)*

Christine Duncan, Boston Children's Hospital (United States) and Harvard Medical School (United States); Anna N. Yaroslavsky, Univ. of Massachusetts Lowell (United States); Wendy London, Dana-Farber Cancer Institute, Harvard Medical School (United States); Amy Juliano, Massachusetts Eye and Ear Infirmary, Harvard Medical School (United States); Stephen Sonis, Biomodels, LLC. (United States); Ather Adnan, Brigham and Women's Hospital, Harvard Medical School (United States); Nathaniel Treister, Brigham and Women's Hospital (United States)

Background: Oral mucositis (OM) is a painful consequence of myeloablative hematopoietic cell transplantation (HCT). Extraorally delivered photobiomodulation therapy (PBMT) is a promising novel intervention for the prevention of OM in children.

Objectives: With funding from an NIDCR R34 planning grant, the objectives of this study are 1) to model the dosimetry of external PBMT and the optimal device parameters for the planned clinical trial, and 2) to plan and design a placebo-controlled Phase 2 multicenter clinical trial to determine whether extraorally delivered PBMT can reduce the duration of severe OM in children, with intent for implementation under subsequent U01 funding.

Methods: External PBMT dosimetry will be evaluated using pediatric head and neck MRI studies to obtain serial measurements of different tissues that will then be used to develop a sophisticated computational model. We plan to conduct a placebo-controlled Phase 2 multicenter clinical trial in which patients 4 to 21 years of age will be randomized 1:1:1 to receive external PBMT dose 1x, external PBMT dose 2x, or sham PBMT starting from conditioning, daily until day +20 post-HCT.

Significance: Extraorally delivered PBMT is a feasible, potentially efficacious intervention that could improve the quality of life for all children undergoing myeloablative HCT. The planned Phase 2 study, based on rigorous dose modeling and with detailed attention to uniform delivery of therapy and OM assessments, will provide critical efficacy data and the potential basis for a subsequent definitive Phase 3 trial.

10048-7, Session 2

The use of laser phototherapy in the management of trigeminal neuralgia pain: two decades of clinical experience

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Trigeminal Neuralgia is one of the most painful conditions known and considered incurable treated by drugs and surgery. Our team has been using phototherapy as a treatment option. The records of 334 patients suffering from Trigeminal Neuralgia were reviewed. Mean ED per session was 17.3 J/cm2; mean number of sessions was 10. The mean treatment ED was 173.2.7 J/cm2. At the end of the treatment 84.4% of patients were asymptomatic or had improvement of the condition, 11.4% remained symptomatic and 4.2% abandoned or did not started the treatment. It is concluded that the use of phototherapy reduced Trigeminal neuralgia pain.

10048-8, Session 2

Biomedical, translational and clinical research on PDT of TMJ

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Introduction Many authors pay particular attention to the therapeutic effect of PDT and laser acupuncture (LA) for the treatment of chronic disorders in the TMJ. The data in world literature, it is clear that the main problem is the dosage ("Dosage is KEY "). Up to this moment has confirmed the statement that because of poliethyologic nature and the wide variety of symptoms in the TMJ disorders is advisable to combine different methods of PDT, depending on the objective condition of the patients and their individual optical characteristic. In recent years there has dictum: "laser + TENS + magnet = success."

The aim of our original biomedical, translational and clinical study was to analyse the real effect of combined therapeutic program in depth and basing on our clinical observations to suggest new approach guaranteeing high therapeutic efficacy of TMJ Photodynamic therapy.

Material and methods We applied the method of building models of clinical situations and then classified them into categories. We studied electromyographic activity, energy metabolism and the state of increased activity of masseters, as well as TMD etiology, diagnosis and therapy, Acugraph meridian energy analysis. Methods for TMJ treatment, approbated by us: LELT +TENS , PIFBM, LA and TENS.

Results and conclusion Laser-assisting treatment of TMJ disorders has a high degree of therapeutic efficacy and can be applied widely in daily dental practice. Best results can be obtained by the combined processes of laser photobiomodulation.

10048-9, Session 2

Photobiomodulation therapy for chemotherapy-induced peripheral neuropathy

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With remarkable advances in the diagnosis and management of cancer in recent years, modern medical science has led to significant growth in cancer survivorship, with greater emphasis on chronic and late complications of various cancer therapies. While acute complications of therapy may necessitate treatment interruptions, dose modifications, or changes in treatment regimens that can significantly affect the effectiveness of treatment and outcomes, chronic, late, and long-term complications of therapy can deleteriously affect quality of life (QOL) of patients in cancer survivors. Chemotherapy-induced peripheral neuropathy (CIPN) is associated with the cumulative dose of delivered chemotherapy and a major cause of compromised quality of life in cancer survivors. There is limited understanding of the etiopathological basis of CIPN, and as such there have been limited available management strategies for prevention or treatment of CIPN, making this a significant unmet need in the field of oncology. There is increasing evidence supporting the use of a non-invasive treatment modality termed photobiomodulation (PBM, previously referred to as "low level light/laser therapy", or LLLT), using low dose biophotonics, to effectively prevent or reverse the biological effects that mediate CIPN. This position paper outlines the available scientific evidence for the application of PBM in the clinical management of CIPN in supportive oncology care.

10048-10, Session 3

Clinical translation of photobiomodulation therapy using evidences from precision molecular pathway analyses *(Invited Paper)*

Praveen Arany D.D.S., Univ. at Buffalo (United States)

Can 'light' be a Drug? To satisfy this definition as a pharmaceutical agent, light must be absorbed and change bodily function. Much evidence from our understanding of our visual cycle and Vitamin D metabolism have all noted this phenomenon. Advances in optophotonic technologies along with a better understanding of light-tissue interactions, especially in in vivo optical imaging and optogenetics, are spearheading the popularity of biophotonics in biology and medicine. The use of lasers and light devices at high doses in dermatology, ophthalmology, oncology and dentistry are now considered mainstream for certain clinical applications such as surgery, skin rejuvenation, ocular and soft tissue recontouring, anti-tumor and anti-microbial photodynamic therapy. In contrast, therapeutic use of low dose biophotonics devices is called Low Level Light / Laser Therapy (LLLT), now termed Photobiomodulation (PBM) Therapy. This therapy is defined as a non-thermal use of non-ionizing forms of electromagnetic radiation to alleviate pain, inflammation, modulating the immune responses and promoting wound healing and tissue regeneration. Surprisingly, despite vast volumes of scientific literature from both clinical and laboratory studies noting the phenomenological evidences for this innovative therapy, limited mechanistic insights have prevented the development of rigorous, reproducible clinical protocols. This presentation will outline our current efforts at ongoing efforts in our group to assess molecular pathways and precisely define clinical treatment variables to enable clinical translation with PBM therapies.

10048-11, Session 3

The biphasic light-dose dependence of spin-adduct EPR signals

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ABSTRACT.

Biphasic behavior of reactive oxygen species (ROS) production as a function of visible (red) light fluence has been reported [1]. Similar results were also reported in the presence of near IR radiation [2]. Using the spin traps DMPO or TEMP as detectors of ROS and an aqueous solution of the methylene blue (MB+) as photosensitizer, we have measured the intensity of the characteristic EPR quartet of the DMPO-OH spin adduct or the characteristic EPR triplet of the TEMPO spin adduct as a function of the light fluence. For both spin traps the observed EPR signal intensity of the respective spin adducts showed a biphasic behavior as a function of the light fluence. Adding the singlet oxygen scavenger sodium azide, we found that both the DMPO-OH and the TMPO EPR signals completely disappeared. This observation together with the fact that irradiation of the MB+ solution in the presence of TEMP, the characteristic spin trap of singlet oxygen, produced the TEMPO triplet EPR signal, clearly indicates that the ROS obtained in the photo-chemical reaction was singlet oxygen. Under the conditions our experiments were carried out, a biphasic ROS production would lead only to an initial increase of the EPR signal with increasing fluence and then the signal should level off but not decrease, as observed. The decrease of the EPR signal after an initial increase can only be explained by assuming that the spin adduct DMPO-OH, or the spin adduct TEMPO which are free radicals, after their initial generation by the oxidation of DMPO or TEMP by singlet oxygen, are themselves oxidized by singlet oxygen. Thus the decreasing part of the biphasic behavior of the EPR signal is not due to a decrease in singlet oxygen production but to the degradation of the spin adduct by singlet oxygen [3].

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[2] Y.-Y. Huang, A. C.-H. Chen, J. D. Carroll, M. R. Hamblin Biphasic Dose Response in Low-Level Light Therapy. Dose Response 7: 358-383 (2009).

[3]P. Bilski. K. Reszka. M. Bilska and C. F. Chignell. Oxidation of the Spin Trap 5,5-Dimethyl-l-pyrroline N-Oxide by Singlet Oxygen in Aqueous Solution, J. Am. Chem. Soc.118, 1330-1338 (1996).

10048-12, Session 3

Photobiomodulation of the oxygen consumption measured in vivo and in real time in the chick's chorioallantic membrane

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The main objective of this project was to develop a new method to study the PBM of the oxygen consumption in vivo and in real time. One interest of this method is that it enables to explore and optimize conveniently different parameters (irradiance, wavelengths, etc) at play in the PBM of the oxygen consumption. This method is based on the use of aminolevulinic acid (ALA) administered to the chick's chorioallantoic membrane (CAM) model, confined in an airtight chamber, to induced protoporphyrin IX (PPIX) as molecular probe of the oxygen partial pressure (pO2). PPIX is of high interest for such measurements since: 1) ALA is an approved precursors; 2) PPIX production takes place in the mitochondria, an organelle playing a key role in the cell respiration. Since PPIX diffuses in different cellular and tissue compartments after its endogenous production, the pO2 can be determined

at different (sub-) cellular/tissue locations; 3) the lifetime of its triplet state is very sensitive to pO2 variations. This enables measuring the pO2 by time-resolved spectroscopy of its delayed fluorescence.

We will present a study, based on this model, to explore the potency of different wavelengths and irradiances to modulate the oxygen consumption by PBM. These results will be compared with other study of PBM effects we have reported with other systems [Vos et al., Plos One, 8(11), 2013; Oueslati et al. PloS One, 10(10), 2015]. Inducing this modulation with different wavelengths, combined or not, provides information of fundamental interest about the light absorbers involved in PBM.

10048-13, Session 4

Low-level light treatment ameliorates immune thrombocytopenia *(Invited Paper)*

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Thrombocytopenia is a common hematologic disorder that is managed primarily by platelet transfusions. We have recently reported in Science Translational Medicine that noninvasive whole body illumination with near infrared laser at a specific setting completely cures acute thrombocytopenia triggered by ?-irradiation within two weeks in mice, as opposed to a 5-week recovery time required in controls. The low-level laser (LLL) also greatly accelerated platelet regeneration in the presence of a common chemotherapeutic drug 5-fluororacil and prevented a severe drop of platelet counts caused by the drug. Mechanistically, LLL stimulated mitochondrial biogenesis specifically in megakaryocytes owing to polyploidy of the cells. LLL also protected megakaryocytes from mitochondrial injury and apoptosis under stress. The multifaceted effects of LLL on mitochondria vigorously bolstered megakaryocyte maturation, robustly facilitated elongation, branching, and formation of proplatelets, and doubled the number of platelets generated from individual megakaryocytes. LLL-mediated platelet biogenesis depended on megakaryopoiesis and was inversely correlated with platelet counts, which kept its effect under the check of plasma thrombopoietin and effectively averted thrombosis even after repeat uses, in sharp contrast to all current agents that stimulate the differentiation of megakaryocyte progenitors from hematopoietic stem cells independently on platelet counts. In the present study, we extended the therapeutic potential of this novel technology to immune thrombocytopenia and showed that additional lasers exhibited similar effects. This safe, drug-free, donor-independent modality represents paradigm-shifting innovation for treatment and primary or secondary prophylaxis of thrombocytopenia.

10048-14, Session 4

Laser-induced immune modulation inhibits tumor growth in vivo

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Photobiomodulation stands as a recommended therapy for oral mucositis induced by oncological therapies. However, its mechanisms of action and, more importantly, its safety in cancer patients, are still unclear.

We assessed cancer cell metabolism and proliferation in vitro and in vivo after exposure to different laser protocols. We exploited both ectopic melanoma and a more physiological oral carcinogenesis mouse model, followed by molecular, histological and immunohistochemical characterization.

Laser irradiation resulted in a slightly increase in cell metabolism and proliferation in vitro, albeit each protocol exerted a difference response. Of notice, in vivo laser light reduced tumour growth and invasiveness, indicating e beneficial effect on tumor microenvironment. Laser-treated tumors were surrounded and infiltrated by immune cells, mainly lymphocytes and dendritic cells, paralleled by an enhanced secretion of type I interferons. In contrast, the number of pro-angiogenic macrophages was reduced in response to laser irradiation, with consequent normalization of the tumor vasculature. Based on these finding we have also started exploring the effect of photobiomodulation on lymphocyte response in an experimental model of vaccination. Preliminary data indicate that laser light induced antigen-specific CD8+ and CD4+ T cell responses.

In conclusion, our data point toward photobiomodulation as an effective strategy to boost the immune response in vivo, with relevant, therapeutic activities in both cancer and vaccination experimental models. These results support the safe use of laser light on cancer patients and open the way to innovative therapeutic opportunities.

10048-16, Session 4

3D Monte Carlo simulation of light propagation for laser acupuncture and optimization of illumination parameters

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Laser acupuncture refers to the effective photochemical and nonthermal stimulation of traditional acupuncture points with low-intensity laser irradiation. Laser acupuncture is painless, sterile, and safe compared to traditional acupuncture. The use of LED devices for phototherapy is a good choice as the laser source for this technology, and the wavelength of LED is taken into account during 600~800nm by MonteCarlo method using MCVM soft. The optical power is the most crucial factor for safety, and the relevant factor is the time for irradiation. Taking these into consideration, a great many researches and investigations are made in this project. In order to protect viability of cells and tissue, and get better therapeutic effect, it's best to control the output power varied at 10mW~30mW range. As to the irradiation time, which is influenced by optical parameters, especially in this study, it's about 10 minutes every time to irradiate. This project focuses on simulating the real environment where traditional acupuncture give treatment of pain, so the factors other than the above three, temperature, age, gender and so on, also should be taken into account. However, this study gives the three factors not only they are the most important factors to affect the effect of laser acupuncture, but also it is more difficult to control than the others. Promising futures for the treatment of laser acupuncture should take more factors into study and the clinical effectiveness would be fed back to the theory giving more information to consult.

10048-17, Session 4

Effect of LED phototherapy on blood lactate level in Taekwondo contest

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The goal of this study was to determine the effect of light-emitting diode phototherapy at 660 nm and 840nm on blood lactate level in the muscle after a Taekwondo contest. Taekwondo is a martial art which is very dynamic with active movements of foot skills so that it leads to the quick generation of lactate in the muscle. Fast decrease of lactate level is important for the

muscle recovery from fatigue in Taekwondo contest.

Eight healthy young male players were included in the trial. Taekwondo contest was arranged to have 3 rounds of 2 minutes competition with 1 minute rest between rounds. And the contest was also arranged to increase exercise intensity level gradually over 85% level of maximal heart rate (HRmax).

15cm x 30cm red and near infrared LED pad with its irradiance of 10mW/cm2 was applied for 10 minutes to brachial muscle and quadriceps muscle of thigh before the Taekwondo contest for one group of players and after the contest for the other group. Blood samples from the players were taken at 5 minutes and 10 minutes after the 3 round contest.

The test results showed that the LED phototherapy application before and after the contest had a significant effect on the decrease of blood lactate level. It was found that the blood lactate level decreased more rapidly from the group of players with LED phototherapy application before the contest than from the group of players with LEDT application after the contest.

10048-18, Session 4

Effects of photobiomodulation therapy (pulsed LASER 904 nm) on muscle oxygenation and performance in exercise-induced skeletal muscle fatigue in young women: a pilot study

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Background: Photobiomodulation therapy (PBMt) has been used to increase muscle performance and improve recovery when applied before exercise. Our aim was to evaluate the effects of PBMt using LASER on muscle oxygenation and muscle performance.

Method: The study was a randomized, participant and assessor-blinded, within-subject crossover trial with placebo control. Procedures were approved by Griffith University Human Research Ethics (Approval No.: 2016/026). Five physically active young women were randomly assigned to either placebo, or active PBMt (12 diode cluster probe; 904 nm; 60 mW; 250 Hz; 43.2 J per site, 129.6 J total) in contact over rectus femoris (RF) muscle of the dominant limb immediately before an isokinetic fatigue protocol. A one-week wash-out period preceded cross-over. A range of isokinetic performance measures were evaluated as well as electromyography. Absolute concentrations of deoxygenated haemoglobin and myoglobin (deoxy[Hb + Mb]) of the RF, an index of local microvascular fractional O2 extraction, was monitored continuously by near-infrared spectroscopy (NIRS). Total haemoglobin concentration as an indicator of microvascular haematocrit was calculated as the sum of the deoxy[Hb + Mb] and oxy[Hb + Mb] signals.

Results: PBMt pre-conditioning reduced time to peak torque when compared to placebo ($P<0.05$). PBMt resulted in a noticeably reduced trend in deoxy[Hb + Mb] during exercise compared to placebo ($P>0.05$).

Conclusion: PBMt before exercise improves indicators of muscle performance, potentially by increasing local matching of bulk and microvascular O2 delivery relative to skeletal muscle O2 utilisation. Further work is required to understand the effect of PBMt on haemodynamic and metabolic characteristics of muscle.

10048-25, Session 4

Introducing: photobiomodulation by low energy chromophore-induced fluorescent light

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Light is the major source of energy photosynthesizing organisms and is sensed by specialized cells in other structures as rods, cones and retinal ganglion cells. Biomedical research has recently shown that photon perception also occurs in what is thought to be non-photosensitive tissue cells. Endogenous chemicals, as flavins, carotenoids and heme, are able to perceive photons and represent the photoreactive sites of larger photoreceptor molecules. Human photoreceptors include cytochrome c oxidase, cryptochromes, and opsin family proteins, which are widely expressed in different cells types.

Light and photoreceptors interactions induce specific signal transduction pathways that recruit transcription factors activating genes involved in multiple aspects of cell biology. The possibility to use visible light to trigger non-thermal, non-cytotoxic, biological reactions through photochemical events has been defined as photobiomodulation. Physiological and subsequent, therapeutic effects of photobiomodulation have been explored in several tissues and cell types, using various low level energy light sources, include low-level laser, light-emitting diodes (LED) and broad band visible light lamps.

A novel state of the art approach to induce photobiomodulation in different cultured human cells, is the use of weak fluorescence emission energy produced by specialized chromophores excited by a narrow bandwidth LED lamp. Unique chromophore emissions in resulting from excitation with narrow bandwidth blue light was studied on Dermal Human Fibroblasts (DHF), wounded 3D skin model (EpiDerm full thickness tissue) and macrophages.

Total collagen production and secretion were significantly up regulated in treated DHF in comparison to untreated control cells, and blue light treated cells. Likewise cell proliferation was significantly enhanced in comparison. The majority of analyzed pro-inflammatory cytokines and chemokines were significantly down regulated in DHF, 3D skin and macrophages. In DHF and 3D skin, the production of specific growth factors such as VEGF and angiogenin was significantly increased. Conditioned medium from treated 3D skin inserts induced a significant increase of tube formation compared to conditioned medium from both untreated and only blue light treated samples, documenting biological effect of treatment dependent growth factors.

In conclusion specialized chromophores excited by a narrow bandwidth LED lamp to emit fluorescent light in a specific wavelengths spectra, influence in vitro the cell shape, cell proliferation, and production of major proteins involved in several skin healing process. These demonstrated effects could lead to reinforcement and revitalization of the skin organization and structure and thus have a major clinical importance.

10048-26, Session 4

Photobiomodulation (PBM) with high-power 640 nm beam: pre-clinical results and propagation model

Denis Julien Gendron, Invitalize (Canada)

A novel treatment modality for photobiomodulation at 640-nm in water window spectrum is introduced, which is termed High Intensity Physio Light (HIPL) Therapy™. This report exemplifies the efficacy performance of this method with three clinical cases: (i) sport injury in the ankle, (ii) bone fractures in the foot, and (iii) painful arthritic shoulder. In all cases treated, a highly efficient pain reduction was systematically experienced by the

patients. This report describes the power, intensity and total dose deposition at target tissue location for each treatment.

(i) Treatment (one-time) for sport injury in the ankle resulted in efficient pain reduction.

(ii) Treatment series (twice weekly) for bone fractures in the foot (one sesamoid and two avulsion fractures) resulted in immediate swelling and pain reduction and continued pain reduction and healing after the 6-week long program

(iii) Treatment series (thrice weekly) for the arthritic shoulder resulted in complete pain reduction after the first 6-week period, and a complete healing of the joint after the 10-week long program.

The arthritic shoulder had developed calcium deposits in joint during the 6-month period prior to experimental therapy, while the patient was under pain-killer treatment. The origin of the degenerative process originated in a subacromial impingement that had developed into a frozen shoulder.

These treatments used a Red Activator Lamp product, custom-designed by Claire Lasers Corporation for clinical research at invitalize. Based on an extensive literature study, the specific conditions used in this experimental work is found unique and we observed for the first time.

Based on a standard model for light-scattering propagation, it is not expected that significant power penetrates at depth relevant to musculoskeletal therapy. A dynamic propagation model is presented to explain the result, and it also suggests further studies to better understand the complex interaction of living tissues with red light.

10048-19, Session PSun

Challenges of transcutaneous laser application for the potential of photobiomodulation of the spinal cord at the scale of a large companion animal

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Photobiomodulation (PBM) has shown to promote axonal regeneration and functional recovery in the spinal cord in rodent models. In contrast, the transcutaneous use of PBM to treat spinal cord diseases of companion animals of varying sizes and body conditions is challenging. This exploratory study examined the light penetration to the spinal canal via surface application of clinically acceptable surface power, as compared to transcranial delivery, on a 40 pound cadaverous dog. The irradiance penetrated to the T13-L1 level of the spinal canal and the cranium under a 10W surface power over a 1cm diameter beam delivered 1cm from the prepared skin surface was measured by a calibrated miniature photo-diode sensor. Additionally, the skin-to-muscle and skin-to-spine penetrations at the T12-13, T13-L1, L1-2, L2-3, and L3-4 levels of the spinal canal were measured by using the photo-diode sensor under a 1W or 10W surface power for indirect estimation of the spinal attenuation. Using a 10W surface power, an irradiance of 145μW/cm2 was measured within the cranium and 130μW/cm2 at the T13-L1 level of the spinal canal. Under a 1W surface power, the skin-to-muscle penetrations at L1-2, L2-3, and L3-4 levels saturated the photo-diode sensor; whereas with a 10W surface power, the skin-to-spine penetrations at the same levels were undetectable. Transcutaneous delivery to the shallowest region of the spinal canal seemed to be comparable to transcranial delivery. Transcutaneous delivery to other levels of the spinal canal is challenging, indicating a potentially narrow tissue window for transcutaneous bio-stimulation of the spinal cord.

10048-20, Session PSun

The use of phototherapy in the management of TMJ pain: clinical evidence of benefits and limitations

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Temporomandibular joint dysfunction painful condition. Despite many treatments being available there is no widely accepted treatment protocol. Lately, phototherapies have also being used for its treatment. The medical records of 303 patients suffering from TMJ pain were reviewed. Mean total ED used per session was 20.3 J/cm2 and mean number of sessions was 10. The mean treatment ED was 203.7 J/cm2. Considering asymptomatic and improved outcomes 78.2% patients showed positive response for the protocol, 17.5% remained symptomatic and 4.9% abandoned/not started the treatment. It is concluded that the use of phototherapy improves the symptoms of TMJ related pain.

10048-21, Session PSun

Evaluation of the efficacy of AmPDT of oral microorganisms with photogem associated to red LED (?640nm±5nm): in vitro

Gustavo M. Pires Santos, Susana Carla P. Sampaio de Oliveira D.D.S., Univ. Federal da Bahia (Brazil); Juliana C. Monteiro, Univ. Estadual de Feira de Santana (Brazil); Aparecida Maria C. Marques D.D.S., Univ. Federal da Bahia (Brazil); Vanderlei S. Bagnato, National Institute of Optics and Photonics (Brazil); Antônio Luiz B. Pinheiro, Univ. Federal da Bahia (Brazil)

This study evaluated using AmPDT (Photogem® + red LED) on microorganisms of the oral cavity. The microorganisms collected and inoculated in TSB medium overnight in culture plates. In 8 wells no Photogem® was used and were irradiated or not controls. The others had Photogem® applied (pre-irradiation time of 5 min) in different concentrations. LED was applied (50 J/cm2) and ELISA and/or turbidity carried out immediate and 1-h later. Using 40 μg/mL of Photogem® + LED irradiation showed best result immediately (36.7%). 1-h later, 5μg/mL + LED irradiation showed best result (42.8%). It is suggested that AmPDT may control oral microorganisms.

10048-22, Session PSun

Investigation on physiological and clinical effects of different light sources in TMJ laser therapy

Julia E. Kamenoff M.D., Medical Univ. Sofia (Bulgaria)

Introduction Laser light Electromagnetic energy has some typical properties for discussions on laser irradiation abilities to control the acute and chronic disorders in TMJ.

Material and Methods During the last six years we have been completed well controlled clinical trials based on the criteria of the American Academy of orofacial pain. The study over the 600 patients (300 women and 300 men), mean age of 47 years have been developed. Patients have been selected on the main clinical sign of TMJ pain and have been divided into four main groups according to the type of PDT method. Based on the action spectra, various wavelengths have been used for TMJ Photodynamic Therapy. Constant dose and time of exposition, as well as various range of frequencies have been applied. In this way the Laser biostimulation response has been directly proportional to the total energy dose, depending of light intensity. Physiological and clinical effects of the followed "active regions"– 660 – 680, 760 – 780, 810 – 830 and 904 – 987 nm have been valued by method of comparative analysis.

Methods applied: LLLT - TENS - red surface laser acupuncture (LA), PIPBM – LA, Laser bioenergetics approach, Complex therapeutic program (CTP).

Results evaluation will be demonstrated by comparative digital ortopantomograph analysis, EEG brain maps, VAS, a metric analysis of the level of the maximum active Mandible opening and EPST through electrophysiological signal evaluation of the patient's body.

10048-23, Session PSun

Low level laser (LED- Ga-Al- As 660) therapy on soft tissue healing: review, mechanism and a case report

Mohammad Nazrul Islam, Shaheed Suhrawardy Medical College and Hospital (Bangladesh)

Background:

Effect of laser on hair Growth of mice (in Hungarian). Mester, E. Szende, B. and Tota, J.G. (1967). Kiserl Orvostud 19. 628-631

Purpose of the work:

The effects of pulsed monochromatic light, with fixed pulsations and wavelengths, on the healing of pressure ulcers were evaluated in this prospective, randomized, controlled study.

Approach and methodology:

Ten patients with 10 bedsore were randomized to receive LLLT or placebo therapy. A placebo-controlled, double-blind study using low energy photon therapy (LLLT) was performed in ten patients with bedsore on the back. Treatment was given three times a week for 10 weeks, using monochromatic (red) optical sources; diode 660nm (GaAlAs-660).

Results:

At the conclusion of the study, the percentage of the initial ulcer area remaining unhealed in the LLLT and placebo groups was 24.4% and 84.7%, respectively (P = 0.0008). The decrease in ulcer area (compared to baseline) observed in the LLLT and placebo groups was 193.0 mm2 and 14.7 mm2, respectively (P = 0.0002).

One patient dropped out of the study, complaining of lack of treatment efficacy; There were no adverse effects.

Conclusions:

In this placebo-controlled, double-blind study LLLT was an effective modality for the treatment of bedsore which were resistant to conventional medical management. The results are encouraging as pulsed monochromatic light increased healing rate and shortened healing time. This will positively affect the quality of life in elderly patients with pressure ulcers.

10048-24, Session PSun

Photonic modulation of EGFR – 280nm low level light arrests cancer cell activation and migration

Claudia M. Botelho, Rogério Marques, Univ. do Minho (Portugal); Viruthachalam Thiagarajan, Bharathidasan Univ. (India); Odete S. Lopes Gonçalves, Henrik Vorum M.D., Aalborg Univ. (Denmark); Andreia Gomes, Univ. do Minho (Portugal); Maria Teresa Neves-Petersen, Aalborg Univ. (Denmark)

Each year 12.7 million people are diagnosed with cancer. Approximately 60% die from it. Cancer-related deaths will increase up to 80% by 2030. Insight into how to control the expression and activity of key proteins that affect cellular proliferation and adhesion is critical for fighting cancer. The Epidermal Growth Factor Receptor (EGFR) has a key role in regulating cell survival, proliferation and migration. Cancer progression is strongly associated with an overexpression of EGFR on the cell surface. EGFR binding to epidermal growth factor (EGF) leads to receptor dimerization, to activation of the intracellular tyrosine kinase domain and to activation of metabolic pathways leading to cell survival and proliferation. EGFR activation is associated with loss of E-cadherin, a protein promoting cellular adhesion on epithelial tissues, inducing epithelial–mesenchymal-transition resulting in cellular dissociation and migration. Low dose UVB light induces 3D structural changes in EGFR impairing EGF-EGFR binding (in vitro studies). We here show that UVB (280nm) illumination of adenocarcinomic human alveolar basal epithelial cells with irradiance levels up to 22 times weaker than the UVB solar output for short periods of time (15-30min) prevents EGF mediated activation of EGFR, halting cellular disaggregation and migration. Filipodia formation and migration has always been observed when non-illuminated cells are activated with EGF. Live cell imaging of the Human A549–EGFR biosensor cell line has been carried out with time-lapse confocal fluorescence microscopy. The new photonic technology disables a key receptor and is applicable to cancer treatment, alone or in combination with other therapies.

Conference 10049: Molecular-Guided Surgery: Molecules, Devices, and Applications III

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10049-1, Session 1

Ultra-fast frequency domain Diffuse Optical Spectroscopy using miniaturized sources and detectors towards quantitative wearables *(Invited Paper)*

Darren M. Roblyer, Alyssa Torjesen, Raeef Istfan, Rachita Chaudhury, Boston Univ. (United States)

Frequency domain Diffuse Optical Spectroscopy (DOS) allows for quantitative extraction of endogenous tissue optical properties and chromophore concentrations. We will present our most recent work on ultra-fast frequency-domain digital DOS which can perform simultaneous six wavelength 50 – 400MHz sweeps at a 97Hz repetition rate, a speed sufficient for capturing hemodynamics of the cardiac cycle in peripheral vasculature. We will show how we are combining fast digital DOS with new multi-wavelength VCSEL sources and miniaturized PMTs and APDs to fabricate frequency-domain wearables towards real-time tracking of quantitative tissue metabolism with applications in oncology, neuroscience, and fitness.

10049-2, Session 1

Depth-resolved, spectroscopic imaging in superficial tissues for quantitative assessment of peripheral vascular health *(Invited Paper)*

David J. Cuccia, Modulated Imaging, Inc. (United States)

No Abstract Available

10049-3, Session 1

Intraoperative fluorescence diffuse optical tomography using spatial frequency domain techniques *(Invited Paper)*

Sang Hoon Chong, Ashwin B. Parthasarathy, Venkaiah C. Kavuri, Univ. of Pennsylvania (United States); Frank A. Moscatelli, Swarthmore College (United States); Sunil Singhal, Hospital of the Univ. of Pennsylvania (United States); Arjun G. Yodh, Univ. of Pennsylvania (United States)

Surgical resection is the most effective treatment strategy for solid tumors, but complete removal of the tumor is critical for post-surgical recovery/long-term survival and is dependent on correct identification of the tumor margin and accurate excision of microscopic residual tumor in the surgical field. Fluorescence image guided surgery is an emerging technique that has shown promise for intraoperative location of tumors and tumor margins. Current versions of such intraoperative fluorescence imaging, however, are generally limited to 2D near-surface images, i.e., without information about tumor depth. Here we present an intraoperative fluorescence imaging system for 3D volumetric imaging of tumors; the system uses spatial frequency domain diffuse optical tomography with an analytic inversion reconstruction method. The new instrument can derive depth-sensitive 3D tumor images at depths up to 1 cm, and it employs compact epi-imaging and illumination suitable for the operating room, with quasi-real-time image reconstruction for surgical visualization. We present experimental results with FDA-approved Indocyanine Green using an extensive array of tissue phantoms and in a pilot in-vivo study.

10049-4, Session 1

Handheld SFDI/polarimetric imaging device for objective evaluation of scars *(Invited Paper)*

Jessica C. Ramella-Roman, Karla Montejo, Nicole Sevilla, Susan Stoff, Mariacarla Gonzalez, Joseph Chue-Sang, Florida International Univ. (United States)

Scars can be debilitating and cause serious functional limitations, significantly reduced physical function and loss of ability to perform normal daily activities. Scar formation is not fully understood and the treatment options have been hampered by the lack of an objective diagnostic tool to assess scars. Presently, assessment of hypertrophic scars has been based on subjective clinician rankings using a four-parameter scale called the Vancouver Scar Scale (VSS) or the Patient Observer Scar Assessment Scale (POSAS) but no objective, standardized tool for quantifying scar severity is available, despite known inadequacies of the subjective scales. We have developed a hand-held multi modal system consisting of a combined Spatial Frequency Domain Imager (SFDI) used for the assessment of tissue molecular components and a polarimeter for structural measurements. The SFDI capability is provided by an Arduino board controlled spectrally and polarimetric diverse Light Emitting Diodes (LED) ring illuminator. For SFDI imagery, the LEDs are combined with sinusoidal patterns. A single pattern snapshot SFDI approach is used to observe and quantify the biological components in the scar tissue including: oxygenated and de oxygenated hemoglobin, water, and melanin. The SFDI system is integrated with a reduced Mueller Matrix polarimetric system, whose illumination is also included in the LED's ring, and providing for the assessment of collagen orientation through Mueller Matrix decomposition. The design of the system and experimental work on phantoms will be presented.

10049-5, Session 1

Sub-diffusive spatial frequency domain imaging provides wide-field visualization and quantification of light scattering as an endogenous biomarker for morphological change in tissue

David M. McClatchy III, Thayer School of Engineering at Dartmouth (United States); Elizabeth J. Rizzo, Dartmouth Hitchcock Medical Ctr. (United States); Stephen C. Kanick, Venkataramanan Krishnaswamy, Jonathan T. Elliott, Thayer School of Engineering at Dartmouth (United States); Wendy A. Wells, Dartmouth Hitchcock Medical Ctr. (United States); Keith D. Paulsen, Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

In spatial frequency domain imaging (SFDI), a spatially modulated intensity pattern is projected on to tissue, with the demodulated reflectance having more superficial sensitivity with increasing spatial modulation frequency. With sub-diffusive SFDI, very high (>0.5 mm-1) spatial modulation frequencies are projected yielding sensitivity to the directionality of light scattering with only few scattering events occurring and sub-millimeter penetration depth and spatial resolution. This technique has been validated in a series of phantom experiments, where fractal distributions of polystyrene spheres were imaged, and through a model based inversion, the size scale distribution versus overall density of these particles could be separated and visualized in spatially resolved maps. With sensitivity to localized light scattering over a wide field of view (11 cm x 14 cm), this technique is being translated for the application of intraoperative

breast tumor margin assessment. To test sensitivity to changes in human breast tissue morphology, a cohort of over 30 freshly excised human breast tissue specimens, including adipose, fibroglandular, fibroadenoma, and invasive carcinoma, have been imaged and co-registered to whole specimen histology. Statistical analysis of the distributions of both textual raw reflectance parameters and model based optical properties for each type of tissue will be presented. Furthermore, classification algorithm development and analysis to predicted likelihood of cancer on the surface of the tissue will also be presented. Reflectance maps, optical property maps, and probability likelihood maps of spatially heterogeneous samples with multiple tissue types will also be shown.

10049-6, Session 2

Addressing the challenges of translating laser speckle imaging to the clinic *(Invited Paper)*

Bernard Choi, Beckman Laser Institute and Medical Clinic (United States)

Since the seminal publication in 2001 by Dunn et al., several research groups have adopted laser speckle imaging (LSI) to map blood flow in laboratory studies, with the highest impact made to date in neurobiology. Due to the simplicity of the components involved in a LSI device, scientists and engineers naturally have applied LSI in studies involving human subjects. In this talk, I will describe seminal efforts, from Beckman Laser Institute and from other research groups, designed to translate LSI from the lab to the clinic. I will emphasize the design considerations from an engineering perspective, and give specific examples of efforts to integrate LSI within specific clinics.

10049-7, Session 2

Fluorescence lifetime FRET non-invasive imaging of breast cancer xenografts provides a measure of target engagement in vivo *(Invited Paper)*

Alena Rudkouskaya, Albany Medical College (United States); Nattawut Sinsuebphon, Xavier Intes, Rensselaer Polytechnic Institute (United States); Margarida Barroso, Albany Medical College (United States)

Fluorescence Lifetime Förster Resonance Energy Transfer (FLIM-FRET) is a unique non-invasive imaging platform to monitor and quantify in vivo target engagement in pre-clinical studies. FLIM FRET is a valuable tool in targeted drug delivery due to its nanoscale-range molecular resolution that detects near-infrared labeled ligand binding to dimerized receptors followed by their uptake into cancer cells in vivo. Various imaging platforms, including PET, lack the ability to directly discriminate between unbound and internalized ligands. Since transferrin receptor (TfR) level is significantly elevated in cancer cells compared to non-cancerous cells, transferrin (Tf) has been successfully used in molecular imaging and targeted anti-cancer drug delivery. The dimeric nature of TfR allows for the quantification of Tf internalization into cancer cells by measuring FLIM FRET between receptor-bound Tf donor and acceptor NIR fluorophore pairs, based on the reduction of donor fluorophore lifetime in live mice. We analyzed tumor morphology, the level of expression of TfR, estrogen receptor (ER) and Tf accumulation in human breast cancer tumor xenografts. We found a remarkable heterogeneity of breast cancer tumors regarding their size, cell density, TfR and ER expression and Tf uptake. The results of this study confirm a strong correlation between in vivo NIR FLIM FRET and ex vivo evaluation of Tf uptake into tumor tissues, thus validating FD% as a robust measure of the target engagement of TfR-Tf in tumor cells in vivo.

10049-8, Session 2

Fluorescence lifetime technique for surgical imaging, guidance and augmented reality

Laura Marcu, Univ. of California, Davis (United States)

The surgeon's limited ability to accurately delineate the tumor margin during surgical interventions is one key challenge in clinical management of cancer. New methods for guiding tumor resection decisions are needed. Numerous studies have shown that tissue autofluorescence properties have the potential to asses biochemical features associates with distinct pathologies in tissue and to distinguish various cancers from normal tissues. However, despite these promising reports, autofluorescence techniques were sparsely adopted in clinical settings. Moreover, when adopted they were primarily used for pre-operative diagnosis rather than guiding interventions. To address this need, we have researched and engineered instrumentation that utilizes label-free fluorescence lifetime contrast to characterize tissue biochemical features in vivo in patients and methodologies conducive to real-time (few seconds) diagnosis of tissue pathologies during surgical procedures. This presentation overviews clinically-compatible multispectral fluorescence lifetime imaging techniques developed in our laboratory and their ability to operate as stand-alone tools, integrated in a biopsy needle and in conjunction with the da Vinci surgical robot. We present pre-clinical and clinical studies in patients that demonstrate the potential of these techniques for intraoperative assessment of brain tumors and head and neck cancer. Current results demonstrate that intrinsic fluorescence signals can provide useful contrast for delineation distinct types of tissues including tumors intraoperatively. Challenges and solutions in the clinical implementation of these techniques are discussed.

10049-9, Session 2

Evaluation of semi-automated method for coregistering fresh tissue fluorescence images with histopathology slides for preclinical evaluation of molecular guided surgery agents

Jonathan T. Elliott, Niki N. Tselepidakis, Kayla A. Marra, Thayer School of Engineering at Dartmouth (United States); Kimberley S. Samkoe, Dartmouth Hitchcock Medical Ctr. (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

The evaluation of new imaging agents for fluorescence guided surgery (FGS) depends on accurately assessing contrast levels in tumor margins, bulk tumor, and necrotic core, since the ability to detect dye in these specific regions will contribute differently to overall clinical value. One of the biggest challenges to properly assessing the regional levels of contrast is in coregistering fluorescence imaging data obtained on fresh tissue with the corresponding formalin-fixed thin slices that are used for tumor grading and immunohistochemical staining. The main reason for this is deformation that results from dehydration during fixation. Within an organ, and even within a tumor, the severity of this deformation can vary with edema, necrosis, and tissue ultrastructure, making the spatial transformation between the fresh tissue images and the micrographs non-rigid and complex. To optimize a non-rigid image coregistration procedure, we imaged rat brain slices with gliomas containing uniform grids of dots which were produced experimentally by inserting ink-coated needles into whole ex vivo brains before slicing. The ink dots are observable on the fresh tissue fluorescence images as well as on the fixed pathology slides providing ground truth features to evaluate coregistration algorithms. A multi-step semi-automated procedure with which the user provides control points corresponding to non-fluorescent landmarks for rigid corregistration, and then computes a non-rigid deformation of the tumor region through expansion was evaluated against the ink points for accuracy.

10049-10, Session 2

Combined spectral and temporal multiplexing for simultaneous color and multispectral fluorescence imaging using a dual sensor approach for intraoperative imaging

Nikolas Dimitriadis, Bart?omiej Grychtol, Martin Theuring, Nikolaos C. Deliolanis, Fraunhofer-Institut für Produktionstechnik und Automatisierung (Germany)

Imaging multiple fluorescent components of biological samples has driven research, drug development and clinical diagnostics throughout the last decades. Though, the multi-component imaging in an intraoperative scenario still exhibits numerous challenges. The fluorescence recording needs to be merged with conventional color imaging, the setup needs to be robust and spatially confined at the same time and additionally the imaging pipeline should run in real-time.

Even though multispectral real-time fluorescence and reflectance imaging is widely spread in biomedical laboratories, its implementation in intraoperative imaging exhibits numerous challenges.

We present a novel imaging approach that combines spectral and temporal multiplexing using complementary multiband filters and two color sensors for the detection of both, fluorescence and color images. Using standard color sensors is highly desirable to acquire surgical reflection images with accurate color representation. Extending the imaging concept by spectral and temporal multiplexing allows the recording of 6 fluorescence and 6 reflectance channels in the visible and near infrared range. The system has the advantage to be easily adaptable to existing surgical imaging equipment and can record at HD video rate without changing filters or any other mechanical parts.

We present the system concept together with a spectral simulation predicting the system quantum efficiency and the expected fluorescence signal in the individual channels. An analysis of these simulation results predicts the signal to noise ratio of each fluorescent component after unmixing. Optimizing the signal to noise ratio aids the selection of an ideal dye, filter of sensor for the desired imaging application. The simulation results will be validated with phantom experiments of different fluorescent dyes.

10049-11, Session 3

Standardization of fluorescence measurements in the UV/vis/NIR/IR *(Invited Paper)*

Ute Resch-Genger, Jutta Pauli, Katrin Hoffmann, Christian Würth, Thomas Behnke, Bundesanstalt für Materialforschung und -prüfung (Germany)

Photoluminescence techniques are amongst the most widely used tools in the life sciences, with new and exciting applications in medical diagnostics and molecular imaging continuously emerging. Advantages include their comparative ease of use, unique sensitivity, non-invasive character, and potential for multiplexing, remote sensing, and miniaturization. General drawbacks are, however, signals, that contain unwanted wavelength- and polarization contributions from instrument-dependent effects, which are also time-dependent due to aging of instrument-components, and difficulties to measure absolute fluorescence intensities [1]. Moreover, scattering systems require special measurement geometries [2] and the interest in new optical reporters with emission > 1000 nm strategies for reliable measurements in the second diagnostic for the comparison of material performance and the rational design of new fluorophores with improved properties [3].

Here, we present strategies to versatile method-adaptable liquid and solid fluorescence standards for different fluorescence parameters including traceable instrument calibration procedures and the design of integrating sphere setups for the absolute measurement of emission spectra and quantum yields in the wavelength region of 350 to 1600 nm [4,5]. Examples are multi-emitter glasses, spectral fluorescence standards, and quantum yield standards for the UV/vis/NIR.

10049-12, Session 3

The use of fluorescence tissue phantoms to standardize responsivity of different imaging systems

Maritoni Litorja, National Institute of Standards and Technology (United States)

Fluorescent tissue phantoms are useful constructs in tracking the daily performance of a fluorescence imaging system. However, fluorescence imaging systems vary according to intended use, such as with an endoscope, or a camera with wide field optics. They also vary in terms of spectral bandwidth, or the sensor. We present a method on how the fluorescence measurement results of a calibrated tissue phantom from two different fluorescence imaging systems can be compared. This demonstrates how tissue phantoms, when calibrated with units of optical radiance, can be used beyond a single optical system.

10049-13, Session 3

Setup for testing cameras for image guided surgery using an controlled NIR fluorescence mimicking light source and tissue phantom

Rudolf M. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands); Giota Georgiou, Vrije Univ. Amsterdam (Netherlands); Albert J. van der Veen, John H. Klaessens, Vrije Univ. Medical Ctr. (Netherlands)

In the development of new near-infrared (NIR) fluorescence dyes for image guided surgery, there is a need for new NIR sensitive camera systems that can easily be adjusted to specific wavelength ranges in contrast the present clinical systems that are only optimized for ICG.

To test alternative camera systems, a setup was developed to mimic the fluorescence light in an tissue phantom to measure the sensitivity and resolution.

Selected narrow band NIR LED's were used to illuminate a 5 mm diameter circular diffuse plate to create uniform intensity controllable light spot (μW-mW) as target/source for NIR camera's. Layers of (artificial) tissue with controlled thickness could be placed on the spot to mimic a fluorescent 'cancer' embedded in tissue. This setup was used to compare a range of NIR sensitive consumers cameras for potential use in image guided surgery. The image of the spot obtained with the cameras was captured and analyzed using ImageJ software.

Enhanced CCD night vision cameras were the most sensitive capable of showing intensities < 1 μW through 5 mm of tissue. However, there was no control over the automatic gain and hence noise level. NIR sensitive DSLR cameras proved relative less sensitive but could be fully manually controlled as to gain (ISO 25600) and exposure time and are therefore preferred for a clinical setting in combination with wifi remote control.

The NIR fluorescence testing setup proved to be useful for camera testing and can be used for development and quality control of new NIR fluorescence guided surgery equipment.

10049-14, Session 3

Development and characterisation of a brain tumour mimicking protoporphyrin IX fluorescence phantom

Yijing Xie, Cristiana Tisca, William Peveler, Sacha Noimark, Adrien E. Desjardins, Ivan P. Parkin, Sebastien Ourselin, Tom Vercauteren, Univ. College London (United Kingdom)

5-ALA-PpIX fluorescence-guided brain tumour resection can increase the accuracy at which cancerous tissue is removed and thereby improve patient outcomes, as compared with standard white light imaging. Novel optical devices that aim to increase the specificity and sensitivity of PpIX detection are typically assessed by measurements in tissue-mimicking optical phantoms of which all optical properties are defined. Current existing optical phantoms specified for PpIX lack consistency in their optical properties, and stability with respect to photobleaching, thus yielding an unstable correspondence between PpIX concentration and the fluorescence intensity.

In this study, we developed a set of aqueous-based phantoms with different compositions, using deionised water or PBS buffer as background medium, intralipid as scattering material, bovine haemoglobin as background absorber, and either PpIX dissolved in DMSO or a novel nanoparticle with similar absorption and emission spectrum to PpIX as the fluorophore. We investigated the phantom stability in terms of aggregation and photobleaching by comparing with different background medium and fluorophores, respectively. We characterised the fluorescence intensity of the fluorescent nanoparticle in different concentration of intralipid and haemoglobin and its time-dependent stability, as compared to the PpIX-induced fluorescence. We corroborated that the background medium was essential to prepare a stable aqueous phantom. The novel fluorescent nanoparticle used as surrogate fluorophore of PpIX presented an improved temporal stability and a reliable correspondence between concentration and emission intensity. We proposed an optimised phantom composition and recipe to produce reliable and repeatable phantom for validation of imaging device.

10049-15, Session 3

Raman-encoded molecular imaging (REMI) with topically applied SERS nanoparticles for intraoperative guidance of breast cancer lumpectomy

Yu Wang, Soyoung Kang, Jonathan T. C. Liu, Univ. of Washington (United States)

There is a need for an intraoperative technology to identify residual tumors at the surgical margin surface of lumpectomy specimens and to guide their complete removal. A comprehensive imaging technique for accurate tumor detection at these marginal surfaces would significantly reduce the costs, inconvenience, and potential complications associated with multiple surgeries. The imaging of dysregulated cell-surface receptors (i.e. biomarkers) is a potential means of identifying tumors with high specificity. However, due to the high variability in biomarker expression patterns between patients as well as within a single tumor, the ability to visualize a diverse multiplexed panel of cell-surface biomarkers is necessary to accurately diagnose tumors. Here, we demonstrate a Raman-encoded molecular imaging (REMI) technology for the simultaneous imaging of up to 4 biomarkers at the surfaces of fresh tissues following a rapid topical-staining (5-min) and rinse-removal (10-s) protocol with targeted SERS NPs, in which various "flavors" of SERS NPs may be identified by their unique spectral signatures. We have developed biomarker-targeted NPs with high binding affinity/avidity, a convection-enhanced topical staining method, a spectral-imaging system and mechanical devices for the automated staining and raster-scanned imaging of fresh human biopsy shavings with a cocktail of up to 5 SERS NPs (4 targeted and 1 untargeted NP). A ratiometric method is employed to eliminate nonspecific effects and to quantify the biomarker expressions. Correlation studies with histopathology indicate that REMI is capable of comprehensively imaging large biopsy shavings (>4 cm2) under time-constrained intraoperative conditions with sub-millimeter resolution for the detection of small residual tumors.

10049-16, Session 4

Clinical fluorescence imaging systems *(Invited Paper)*

John Fengler, Novadaq Technologies, Inc. (Canada)

No Abstract Available

10049-17, Session 4

Augmented reality with Microsoft Hololens holograms for near infrared fluorescence based image guided surgery

Nan Cui, Pradosh Kharel, Viktor Gruev, Washington Univ. in St. Louis (United States)

Near infrared fluorescence (NIRF) based image guided surgery aims to provide vital information to the surgeon in the operating room, such as locations of cancerous tissue that should be resected and healthy tissue that should to be preserved. Targeted molecular markers, such as nerve targeted or tumor targeted, are used in conjunctions with NIRF imaging and display systems to provide key information to the operator in real-time. One of the major hurdles for the wide adaptation of these imaging systems is the high cost to operate the instruments, large footprint and complexity of operating the systems. The emergence of wearable NIRF systems has addressed these shortcomings by minimizing the imaging and display systems' footprint and reducing the operational cost. However, one of the major shortcomings for this technology is the replacement of the surgeon's natural vision with an augmented reality view of the operating room. In this paper, we have addressed this major shortcoming by exploiting hologram technology from Microsoft Hololens to present NIR information on a color image captured by the surgeon's natural vision. NIR information is captured with a CMOS sensor with high quantum efficiency in the 800 nm wavelength together with a laser light illumination light source. The NIR image is converted to a hologram that is displayed on Microsoft Hololens and is correctly co-registered with the operator's natural eyesight. The system has been successfully used in preclinical studies on tumor bearing mice with tumor targeted NIR markers for complete resections of all cancerous tissues.

10049-18, Session 4

Open-air multispectral fluorescence-guided surgery platform for intraoperative detection of malignant tissue under ambient conditions

Ali Behrooz, Kristine Vasquez, Peter A. Waterman, Jeffrey D. Peterson, Jeff Meganck, Peter J. Miller, Josh Kempner, PerkinElmer, Inc. (United States)

Intraoperative resection of tumors currently relies upon the surgeon's ability to visually locate and palpate tumor nodules. Undetected residual malignant tissue often results in the need for additional treatment or surgical intervention. The Solaris™ platform is a multi-spectral open-air fluorescence imaging system designed for translational fluorescence-guided surgery. Solaris supports video-rate imaging in four fixed fluorescence channels ranging from visible to near infrared, and a multispectral channel equipped with a liquid crystal tunable filter (LCTF) for multispectral image acquisition (520-620 nm). Identification of tumor margins using reagents emitting

in the visible spectrum (400-650 nm), such as fluorescein isothiocyanate (FITC), present challenges considering the presence of auto-fluorescence from tissue and food (alfalfa). To overcome this, Solaris acquires LCTF-based multispectral images, and by applying an automated spectral unmixing algorithm to the data, separates reagent fluorescence from tissue and food auto-fluorescence. The unmixing algorithm uses vertex component analysis to automatically extract the primary pure spectra, and resolves the reagent fluorescent signal using non-negative least squares. For validation, intra-operative in vivo studies were carried out in tumor-bearing rodents injected with FITC-dextran reagent that is primarily uptaken in malignant tissue 24 hours post injection. In the absence of unmixing, fluorescence from tumors are not distinguishable from the surrounding tissue. Upon spectral unmixing, the FITC-labeled malignant regions become well defined and detectable. The results of these studies substantiate the multispectral power of Solaris in resolving FITC-based agent signal in tumor masses, under ambient and surgical light, and enhancing the ability to surgically resect them.

10049-19, Session 4

Light-sheet microscopy for rapid 3D digital pathology of fresh tissue specimens

Adam K. Glaser, Nicholas P. Reder M.D., Chengbo Yin, Ye Chen, Univ. of Washington (United States); Lawrence D. True, Univ. of Washington Medical Ctr. (United States); Jonathan T. C. Liu, Univ. of Washington (United States)

For microscopic inspection of fresh tissues obtained during or after surgical resection, or through a biopsy procedure, pathologists must currently cut thick excised tissues into thin sections, a time-consuming and expensive practice that requires the tissues to be chemically fixed or frozen, embedded in wax or a freezing compound, and then mounted and stained on a glass slide. While various optical-sectioning microscopy methods have been proposed as rapid alternatives to physical sectioning, they typically provide an in-focus image of a single flat focal plane with a narrow depth of focus, in which it is difficult to image the irregular surfaces of fresh tissue specimens over a large area unless elaborate tissue-flattening and alignment procedures are utilized. Additional challenges have included insufficient resolution, contrast, field of view, and/or imaging speed, all of which have limited the clinical viability of these prior systems. To overcome these limitations, we have designed an inverted light-sheet microscope (LSM). By separating and decoupling the illumination and collection optics, the system has an additional degree of freedom when compared to conventional single-axis microscopes, which allows for volumetric microscopy over an extended depth of focus, from which the tissue surface can be digitally segmented and displayed. We describe the design, characterization, and imaging performance of a two-color LSM system that can image nuclear and cytoplasmic features of fresh tissue surfaces at high speed (<1 min/cm^2) over a wide-area (up to 10x10 cm) with high resolution (~2 microns) and contrast (a usable imaging depth of ~50 microns).

10049-20, Session 4

Intraoperative visualization of plasmon resonant liposomes using augmented microscopy

Jeffrey R. Watson, Summer Garland, Marek Romanowski, The Univ. of Arizona (United States)

Plasmon resonance associated with nanoparticles of gold enables photothermal ablation of tissues, controlled drug release, or interrogation of individual cells, all with exquisite temporal and spatial control. These technologies may support many applications of precision medicine. However, their clinical implementations will require new methods of intraoperative imaging and guidance. Here we describe application of augmented microscopy in guiding surgical procedures employing plasmon resonant gold-coated liposomes. Plasmon resonances contemplated for biomedical applications often occur in the near-nfrared spectral range, preferred for its better penetration through the biological tissues. In one example, the plasmon resonant gold-coated liposomes we introduced earlier can be designed to interact with the wavelengths in the spectral range extending up to 1200 nm. When activated with a laser beam at the wavelength matching the position of the plasmon resonance, these liposomes release their encapsulated content. Augmented microscopy we demonstrated recently is an intraoperative imaging technique that generates a real-time overlay of the bright field images with the color-coded near-infrared images of the same field of view. In this report we describe how this imaging technique can be used to visualize NIR laser beams and plasmon resonant liposomes within the bright field images of the surgical field. We discuss implementation of femtosecond pulse delivery to activate nonlinear processes in plasmon resonant nanoparticles. We show that plasmon resonant theranostic agents in combination with the augmented microscope provide a novel system for intraoperative imaging of NIR guided drug delivery and therapies.

10049-21, Session 5

Theranostic imaging *(Invited Paper)*

Xinning Wang, Brian Q. Tsui, Gopolakrishnan Ramamurthy, Ping Zhang, Joseph Meyers, Malcolm E. Kenney, Jonathan Kiechle, Lee Ponsky, James P. Basilion, Case Western Reserve Univ. (United States)

Prostatectomy has been the mainstay treatment for men with localized prostate cancer. Surgery, however, often can result in major side effects, which are caused from damage and removal of nerves and muscles surrounding the prostate. A technology that can help surgeons more precisely identify and remove prostate cancer resulting in a more complete prostatectomy is needed. Prostate-specific membrane antigen (PSMA), a type II membrane antigen highly expressed in prostate cancer, has been an attractive target for imaging and therapy. The objective of this study is to develop low molecular weight PSMA-targeted photodymanic therapy (PDT) agents, which would provide image-guidance for prostate tumor resection and allow for subsequent PDT to eliminate un-resectable or remaining cancer cells. Based on our highly negatively charged, urea-based PSMA ligand PSMA-1, we synthesized two PSMA-targeting PDT conjugates named PSMA-1-Pc413 and PSMA-1-IR700. In in vitro cellular uptake experiments and in vivo animal imaging experiments the two conjugates demonstrated selective and specific uptake in PSMA-positive PC3pip cells/tumors, but not in PSMA-negative PC3flu cells/tumors. Further in vivo photodynamic treatment proved that the two PSMA-1-PDT conjugates can effectively inhibit PC3pip tumor progression. The two PSMA-1-PDT conjugates reported here may have the potential to aid in the detection and resection of prostate cancers. It may also allow for the identification of unresectable cancer tissue and PDT ablation of such tissue after surgical resection with potentially less damage to surrounding tissues.

10049-22, Session 5

Development of small molecule protease probes for optical imaging applications *(Invited Paper)*

Matthew Bogyo, Stanford Univ. (United States)

Proteases are enzymes that play pathogenic roles in many common human diseases such as cancer, asthma, arthritis, atherosclerosis and infection by pathogens. Tools to dynamically monitor their activity can be used as diagnostic agents, as imaging contrast agents for intra-operative image guidance and for the identification of novel classes of protease-targeted drugs. I will describe our efforts to design and synthesize small molecule probes that produce a fluorescent signal upon binding to a protease target. We have identified probes that show tumor-specific retention, fast activation kinetics, and rapid systemic distribution making them useful for

real-time fluorescence guided tumor resection and other diagnostic imaging applications.

10049-23, Session 5

Sprayable enzyme-activatable fluorescent probes: kinetic mapping using dynamic fluorescence imaging can help detecting tiny cancer foci.

Hisataka Kobayashi, National Cancer Institute (United States)

Optical fluorescence-guided imaging is increasingly used to guide surgery and endoscopic procedures. Sprayable enzyme-activatable probes are particularly useful because of high target-to-background ratios that increase sensitivity for tiny cancer foci. However, green fluorescent activatable probes suffers from interference from autofluorescence found in biological tissue. Dynamic imaging followed by the kinetic analysis could be detected local enzyme activity and used to differentiate specific fluorescence arising from an activated probe in a tumor from autofluorescence in background tissues especially when low concentrations of the dye are applied to detect tiny cancer foci. Serial fluorescence imaging was performed using various concentrations of ?-glutamyl hydroxymethyl rhodamine green (gGlu-HMRG) which was sprayed on the peritoneal surface with tiny implants of SHIN3-dsRed ovarian cancer tumors. Temporal differences in signal between specific green fluorescence in cancer foci and non-specific autofluorescence in background tissue was measured and processed into three kinetic maps reflecting maximum fluorescence signal (MF), wash-in rate (WIR), and area under the curve (AUC), respectively. Especially at lower concentrations, kinetic maps derived from dynamic fluorescence imaging were clearly superior to unprocessed images for detection small cancer foci.

10049-24, Session 5

Characterizing nerve-specific fluorophores for image-guided nerve sparing surgical procedures

Connor W. Barth, Summer L. Gibbs, Oregon Health & Science Univ. (United States)

Nerve damage plagues surgical outcomes, significantly affecting post-surgical quality of life. Surprisingly, no method exists to enhance direct nerve visualization in the surgical suite, and nerve detection is completed through a combination of palpation and visualization when possible. Fluorescence image-guided surgery offers a potential means of enhanced nerve identification and preservation. Importantly, several classes of fluorescent small molecules have recently been demonstrated to have nerve specificity including the distyrylbenzenes (DSB), select oxazines (oxazine 4 perchlorate), and certain cyanines (3,3'-diethylthiatricarbocyanine iodine). The nerve-sparing radical prostatectomy is a surgical procedure that could benefit from fluorescence image-guided nerve identification. Although the nerve-sparing surgical technique was developed over 30 years ago, nerve damage following radical prostatectomy is reported in some form in up to 60% of patients one to two years post-surgery. To facilitate translation of fluorescence image guided surgery to the nerve sparing prostatectomy, a direct administration methodology was developed that allows selective nerve highlighting with a significantly lower fluorophore dose than systemic administration. Tissue penetration will be critical for clinical feasibility of the direct administration methodology. Nerve-specific fluorophore tissue penetration was modeled and quantified in vivo to predict optimal nerve-specific fluorophores and formulation strategies. In addition, several biomolecular targets of Oxazine 4, a promising candidate for nerve-specific fluorophore development into the near-infrared, have been identified, providing insight into the mechanism of nerve-specificity.

10049-25, Session 5

Evaluation of contrast and intratumoral heterogeneity for ABY-029 in glioma, with pre-doses of unlabeled cetuximab as a receptor 'cold dose'

Ana Luiza Ribeiro de Souza, Thayer School of Engineering at Dartmouth (United States) and CAPES Foundation, Ministry of Education of Brazil (Brazil); Kayla A. Marra, Clare D. Snyder, Jason R. Gunn, Thayer School of Engineering at Dartmouth (United States); Kimberley S. Samkoe, Geisel School of Medicine, Dartmouth College (United States) and Thayer School of Engineering at Dartmouth (United States); Keith D. Paulsen, Thayer School of Engineering at Dartmouth (United States) and Geisel School of Medicine, Dartmouth College (United States); Joachim Feldwisch, Affibody AB (Sweden); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States) and Geisel School of Medicine, Dartmouth College (United States)

Several recent studies have demonstrated the use of intraoperative fluorescent imaging as a strategy to enhance the visual contrast between tumor and normal tissue. ABY029 is a fluorescently labeled EGRF?binding Affibody molecules developed for human use during tumor resection in fluorescence guided surgery. The aim of this study is enhance the contrast tumor-to-normal using either crescent doses of ABY029 or unlabeled cetuximab as a "cold dose" in a xenograft model. For this purpose, nude rats were inoculated with the U251 human glioma cell line in the brain and tumors were allowed to grow for 3-4 weeks. The rats received different doses of ABY?029 and fluorescence ex vivo imaging of brain slices was acquired 1 h post-injection. For the competitive binding assay between ABY029 and cetuximab, U251 cells were incubated for 1 h at 4 °C with either ABY029, unlabeled cetuximab or a mixture of both and the fluorescence was evaluated by flow cytometry. From the ex vivo analysis of the brain, the data showed that the contrast tumor-to-normal brain increased until it reached a plateau with 10X of the microdose of ABY029. The in vitro experiments showed that the cetuximab was able to block ABY029 binding to EGFR. Further studies will be performed to evaluate if the administration of unlabeled cetuximab would be able to increase the contrast tumor-to-normal tissue either in brain or subcutaneous tumor, making the ABY029 a suitable fluorescent probe to be used in fluorescent guided surgery of several tumors overexpressing EGFR.

10049-26, Session 6

Normalization and standardization of fluorescence imaging *(Invited Paper)*

Vasilis Ntziachristos, Helmholtz Zentrum München GmbH (Germany)

Fluorescence imaging has been considered for over a half-century as a modality that could assist surgical guidance and visualization. The administration of fluorescent molecules with sensitivity to disease biomarkers and their imaging using a fluorescence camera can outline pathophysiological parameters of tissue invisible to the human eye during operation. The advent of fluorescent agents that target specific cellular responses and molecular pathways of disease has facilitated the intraoperative identification of cancer with improved sensitivity and specificity over non-specific fluorescent dyes that only outline the vascular system and enhanced permeability effects. With these new abilities come unique requirements for developing methods to calibrate imaging systems and algorithms. The talk discusses the use of fluorescence phantoms employed to validate fluorescence imaging systems and results. We identify

current limitations and discuss the level of phantom complexity that may be required for developing a universal strategy for fluorescence imaging calibration. We also introduce the principles of standardization and reversion for providing accurate standardized readings in fluorescence molecular imaging.

10049-27, Session 6

Overview of regulatory approval paths for optical surgical navigation *(Invited Paper)*

Paula Jacobs, National Cancer Institute (United States)

In this presentation, we will provide an overview of several regulatory aspects of optical surgical guidance with an emphasis on what drugs and devices the FDA has approved and the modern relevance of that approval. .When approving imaging devices and agents for clinical use, the FDA relies heavily on existing data and regulatory precedence. Taking this into account, the field should identify established surrogates of clinical benefit to aid the approval pathways of this promising technology. However, this task is confounded by the lack of contrast-enhanced oncologic agents, leading to a lack of regulatory precedence. Therefore, it is critical to identify parallel pathways using similar modalities and companion agents that can be used as potential regulatory surrogates for FDA consideration.

10049-28, Session 6

From micro to macro: how far NIR imaging technologies can help physicians in clinical decision-making *(Invited Paper)*

Corinne Laplace-Builhé, Muriel Abbaci, Frederic De Leeuw, Ingrid Breuskin, Odile Casiraghi, Peggy Dartigues, Ranya Soufan, Stephane Temam, Malek Ferchiou, Aïcha Ben Lakhdar, Chafika Mazouni, Dana Hartl, Institut Gustave Roussy (France)

New optical imaging tools relaying either on endogenous tissue properties or fluorescent contrast agents have reached the clinical field for more than a decade. Technologies based on the miniaturization of imaging systems such as confocal endomicroscopy and optical coherence tomography (OCT) have been intended to achieve in-vivo "optical biopsies", whereas NIR camera and photoacoustic devices provide gross and functional information in organs. Depending on the reading scale available, these tools are used either to refine the early diagnosis of cancerous lesions, or to improve image-guided surgical procedures. The current adoption of these technologies in the clinic is still limited to just a few applications, such as GI endoscopy, eye and vascular diseases, sentinel lymph node mapping, or the monitoring of flap perfusion in reconstructive surgery. The biophysical changes in pathological tissues and imaging depth requirements, the bioavailability of contrast agents, the need of multiscale imaging, and quantification of fluorescent signals are the main issues that must be considered to improve the overall clinical evaluation of these new tools. They will be discussed here from preclinical and clinical data obtained with endomicroscopy, Full-Field OCT and NIR imaging.

10049-29, Session 6

Clinical application of a multi-modal endoscope with fluorescent peptide in detection of colorectal neoplasia

Zhenzhen Dai, Univ. of Michigan Health System (United States); Bishnu P. Joshi, Zhenghong Gao, Jeonghoon Lee, Navin Ghimire, Anoop Prabhu, Erik J. Wamsteker, Richard S. Kwon, Grace H. Elta, Henry D. Appelman, Scott R. Owens, Rork Kuick, Kim K. Turgeon, Thomas D. Wang, Univ. of Michigan (United States)

Early detection of precursor lesions for colorectal cancer can greatly improve survival. Pre-neoplasia can appear flat with conventional white light endoscopy. Sessile serrated adenomas (SSA) are precursor lesions found primarily in the proximal colon and frequently appear flat and indistinct. We performed a clinical study of n=37 patients using a multimodal endoscopy with a FITC-labeled peptide specific for SSA. Lesions were imaged with white light, reflectance and fluorescence. White light images were acquired before the peptide was applied and were used to help localize regions of abnormal tissues rightly. Co-registered fluorescence and reflectance images were combined to get ratio images thus the distance was corrected. We calculated the target/background ratio (T/B ratio) to quantify the images and found 2.3-fold greater fluorescence intensity for SSA compared with normal tissues. We found the T/B ratio for SSA to be significantly greater than that for normal colonic mucosa with 89.47% sensitivity and 91.67% specificity at the threshold of 1.22. An ROC curve for SSA and normal mucosa was also plotted with area under curve (AUC) of 0.93. The result also shows that SSA and adenoma are statistically significant and can be identified with 78.95% sensitivity and 90.48% specificity at the threshold of 1.66. An ROC curve was plotted with AUC of 0.88. Therefore, our result shows that the application of a multimodal endoscope with fluorescently labeled peptide can quantify images and works especially good for the detection of SSA which is a premalignant flat lesion conferring a high risk of subsequently leading to a colon cancer.

10049-30, Session 7

Image-guided urologic surgery: intraoperative optical imaging and tissue interrogation *(Invited Paper)*

Joseph C. Liao, Stanford Univ. (United States)

Emerging optical imaging technologies can be integrated in the operating room environment during minimally invasive and open urologic surgery, including oncologic surgery of the bladder, prostate, and kidney. These technologies include macroscopic fluorescence imaging that provides contrast enhancement between normal and diseased tissue and microscopic imaging that provides tissue characterization. Optical imaging technologies that have reached the clinical arena in urologic surgery are reviewed, including photodynamic diagnosis, near infrared fluorescence imaging, optical coherence tomography, and confocal laser endomicroscopy. Molecular imaging represents an exciting future arena in conjugating cancer-specific contrast agents to fluorophores to improve the specificity of disease detection. Ongoing efforts are underway to translate optimal targeting agents and imaging modalities, with the goal to improve cancer-specific and functional outcomes.

10049-31, Session 7

Novel applications of near-infrared fluorescence imaging in orthopedic surgery *(Invited Paper)*

Eric R. Henderson, Dartmouth Hitchcock Medical Ctr. (United States); Alisha V. DSouza, Keith D. Paulsen, Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

Sarcomas are cancers of the bones, muscles, nerves, and fat that require complete surgical removal for cure. The primary surgical goal therefore is to remove the tumor with a zone of normal, non-cancerous tissue surrounding the tumor, considered a 'negative' surgical margin.

At present, surgeons rely on radiologic imaging and visual and tactile clues

to gauge cancer depth and guide surgical excision. This can result in removal of too much or too little tissue, which can lead to unnecessary removal of vital structures or incomplete cancer removal, respectively. Both results can have negative effects on ultimate patient outcome, with positive margins reported in 23% of sarcoma surgeries.

Near-infrared (NIR) fluorescence probes are molecules that when stimulated with specific, known frequencies of near-infrared light will emit light of another distinct frequency. NIR light penetrates human tissue reasonably well and therefore can be used to detect the presence and location of unseen structures labeled with NIR fluorescence probes through several centimeters of tissue. Intra-operative near-infrared (NIR) fluorescence probes have been effective for this purpose in brain tumor surgery and may be applicable to sarcoma surgery.

Foundational research is needed to explore the potential of this affibody probe and perfusion probes to estimate margin thickness in sarcoma surgery. In this study we will determine if sarcoma labeling using NIR fluorescence probes is superior with perfusion probes or a novel affibody probe. We will also determine whether NIR fluorescence using perfusion probes or a novel affibody probe can be correlated accurately to margin thickness.

10049-32, Session 7

Image guided surgery using near-infrared fluorescence: road to clinical translation of novel probes for real time tumor visualization *(Invited Paper)*

Charlotte E. S. Hoogstins M.D., Henricus J. M. Handgraaf M.D., Leonora S. F. Boogerd M.D., Jacobus Burggraaf M.D., Alexander L. Vahrmeijer M.D., Leiden Univ. Medical Ctr. (Netherlands)

Due to its relatively high tissue penetration, near-infrared (NIR; 700-900 nm) fluorescent light has the potential to visualize structures that need to be resected (e.g. tumors, lymph nodes) and structures that need to be spared (e.g. nerves, ureters, bile ducts). NIR fluorescence imaging using non-targeted fluorescent probes has been extensively studied in the last decade. Although proven feasible, tumor-specific imaging can be dramatically enhanced using tumor-specific fluorescent contrast agents. Clinical translation of these agents is challenging and hurdles have to be overcome. In this overview we recapitulate the key regulations for first-in-human studies with fluorescent agents, provide insight in different strategies for swift clinical translation and discuss how clinical introduction of these fluorescent agents was achieved.

10049-33, Session 7

Technological advances on fluorescence-guided neurosurgery *(Invited Paper)*

Keith D. Paulsen, Thayer School of Engineering at Dartmouth (United States)

No Abstract Available

10049-50, Session 8

Regulation, procedure, experience and advice about clinical translation of fluorescence-guided surgery

Abbas Bandukwala, U.S. Food and Drug Administration (United States)

No Abstract Available

10049-41, Session PSun

A laparoscopic applicator probe for real-time en-face mapping of near-surface optical sources of heterogeneity over a 1cm instrument-tip-size field-of-view

Daqing Piao, Kenneth E. Bartels, G. Reed Holyoak, Oklahoma State Univ. (United States); Sanjay Patel, The Univ. of Oklahoma Health Sciences Ctr. (United States)

Surgeons operating laparoscopically often have to rely upon subjective visual cues for complete oncological control and avoiding tumor violation or iatrogenic injury to critical tissues. A laparoscopic imaging tool to allow assessment of tumor margin or identification of anatomical structures buried under the layer of tissue being dissected is desirable to probe tissue contrast at a few millimeters depth, visualize over the lateral view for resection guidance, have a non-microscopic field-of-view (FOV) adequate for rapid survey of the resection site, and form the image in real-time. Probing light diffusely propagated through tissue provides sub-surface sensitivity, but the image formation generally involves intense computation that may be costly to intraoperative time-frame. Projecting these modalities laparoscopically to sample subsurface tissue heterogeneity over a non-microscopic FOV for rapid site-survey is also challenging. We demonstrate a laparoscopic applicator probe and a method thereof for real-time en-face mapping of near-surface heterogeneity for potential use towards intraoperative margin assessment. The probe fits a 12mm port and houses 128 copper-coated 750μm fibers that form radially alternating illumination (70 fibers) and detection (58 fibers) channels. By simultaneously illuminating the 70 source channels of the laparoscopic probe that is in contact with a scattering medium and concurrently measuring the light diffusely propagated to the 58 detector channels, the presence of near-surface optical heterogeneities can be resolved in an en-face 9.5mm field-of-view in real-time. Visualization of subsurface margin of strong attenuation contrast at a depth up to 3mm is demonstrated at a frame rate of 1.25Hz.

10049-42, Session PSun

Modular augmented microscopy with spatial light modulation

Summer Garland, Jeffrey R. Watson, The Univ. of Arizona (United States); Nikolay L. Martirosyan M.D., G. Michael Lemole Jr., Banner Univ. Medical Ctr. (United States); Marek Romanowski, The Univ. of Arizona (United States)

Success of surgical removal of brain cancer relies on accurate boundary delineation. Near-infrared (NIR) fluorescent contrast agents may help to improve visualization of tumor boundaries via uptake into tumor tissue. Augmented microscopy allows for simultaneous display of NIR fluorescence images with real images of the surgical field, potentially aiding in improved tumor resection. Here, we present two examples of augmentation modules employing different types of spatial light modulators.

The self-contained augmentation module is designed to simply be installed into a beam splitter position available in surgical stereomicroscopes. For this demonstration, we employed Labomed Prima, a stereoscopic surgical microscope. Excitation is provided by a 780nm NIR light emitting diode (Thorlabs) and near infrared images are collected using a highly sensitive electron multiplication CCD camera C9100-13 (Hamamatsu). The false-colored NIR image is injected into the optical path of the microscope using a miniature projector, a DLP LightCrafter Display 2010 EVM (Texas Instruments). The real and synthetic images were merged using a spatial light modulator, either liquid crystal-based switchable mirror or digital micromirror device. Mechanical designs, including comprehensive light path tracing with integration of SLM technology, was accomplished using a computer-aided design software (SolidWorks). Use of computer-aided design software in connection with CNC manufacturing results in a superior placement of peripheral components vital to this technology.

The use of SLM technology in surgical microscopes may increase the sensitivity of NIR fluorescence detection and enable optical image processing, while providing direct control of the real-time overlay ratio between bright-field and synthetic images.

10049-43, Session PSun

Pre-clinical evaluation of fluorescent ABY-029 in 3 mouse sarcoma models, to assess enhanced contrast in fluorescence guided surgery relative to fluorescent perfusion contrast

Kayla A. Marra, Thayer School of Engineering at Dartmouth (United States); Marissa Murchiss, Univ. of Arkansas (United States); Jason R. Gunn, Brian W. Pogue, Dartmouth College (United States); Eric R. Henderson, Dartmouth Hitchcock Medical Ctr. (United States)

ABY-029 is a fluorescently labeled affibody targeted to epidermal growth factor receptor (EGFR), which is widely overexpressed in a range of neoplasms. Fluorescently labeled targeted proteins could be powerful tools in the field of tumor resection with the potential to provide molecular-originated tumor-to-background contrast, and this agent emits in the 800nm band for near infrared imaging. A recent toxicity study work has shown that injected levels of ABY-029 are safely tolerated all the way up from a microdose to 1000X a microdose level. Work is underway, preparing to enter Phase 0 clinical trials in glioma, sarcoma and head and neck resections. The aim of this specific study was to pre-clinically evaluate the efficacy of ABY-029 in sarcoma models in vivo, prior to clinical use in sarcoma clinical trial patients. The three sarcoma lines (SW 982, SK-NEP-1 and MG-63) were cytometrically evaluated for EGFR expression and subcutaneously grown in mice. Tumor bearing mice were then injected with ABY-029, as well as a matched untargeted fluorescent protein tracer emitting in the 700 nm wavelength band. After three hours of circulation the animals were sacrificed. Tumor, surrounding tissue, as well as normal skin and muscle were removed for ex vivo imaging on a 700/800 channel flatbed scanner. Images were analyzed for specific EGFR uptake (ABY-029), relative to the perfusion contrast (non-specific dye-protein) and the tumor-to-background contrast. We hypothesize that ABY-029 will prove useful in identifying the margins of EGFR positive sarcoma tumors, for real time information during surgical resection.

10049-44, Session PSun

Bio-inspired multi-spectral multi-integration time imager for diabetic and ischemic mice models

Missael Garcia, Mohamed Zayed, Kyoung-mi Park, Viktor Gruev, Washington Univ. in St. Louis (United States)

Evolution has favored arthropods with remarkably elegant imaging systems. An exceptional example of this is the Morpho butterfly which has benefited immensely from the development of photonic structures to form tapetal filters, present in the ommatidia of their compound eyes. Analogous to the way the Morpho butterfly stacks alternating layers of air and cytoplasm to create these spectral filters, we have created pixelated interference filters by alternating nano-metric layers of silicon dioxide and titanium dioxide. This array consists of four different types of filters sensitive to the red, green, blue, and NIR spectra, where each type sits on top of a CMOS pixel. We have combined this spectral technology with a custom CMOS sensor capable of non-destructive and flexible pixel-selection readouts. The imaging sensor weights less than 10 grams, with an effective resolution of 1280-by-720 pixels and a frame rate of 24 FPS. Due to the pixelated spectral topology of our imaging system, the co-registration error among spectral planes is less than 1.4 pixels, while the independent integration time of the spectral planes yields a hundredfold improvement in sensitivity for NIR fluorescent imaging. We also observed that high-resolution in vivo angiography can be performed when the imaging system is combined with intravenous administration of indocyanine green (ICG). Our imaging system has a maximal optical density of 14, which produces a 100 pM detectability. This imaging system can be used to accurately characterize altered dynamic arterial perfusion in a clinically relevant murine model for human critical limb ischemia.

10049-45, Session PSun

Dexamethasone enhances 5-ALA/PpIX contrast but degrades ABY-029 contrast at glioma margins during fluorescence guided resection

Jonathan T. Elliott, Clare D. Snyder, Kayla A. Marra, Scott C. Davis, Thayer School of Engineering at Dartmouth (United States); David W. Roberts, Dartmouth Hitchcock Medical Ctr. (United States); Keith D. Paulsen, Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

Exogenous fluorescent dyes are increasingly being used to guide neurosurgery as part of the fluorescence-guided resection (FGR) paradigm. Approved imaging agents include 5-aminoleuvulinic acid induced protoporphyrin IX (5-ALA/PpIX), indocyanine green (ICG) and fluorescein sodium (FS). Each of these behaves differently in terms of uptake and delivery to tumor cells, and provides contrast based on different underlying mechanisms. However, all are influenced by the local tissue kinetics. Dexamethasone, and other steroids, are common first-line treatment for edema-related symptoms such as headache, nausea and seizures. While prevalence varies with physician and medical center, approximately 36% of adult glioma patients at Dartmouth are on steroid therapy at the time of surgery. Because dexamethasone stabilizes the blood brain barrier, we investigated its influence on contrast-to-background in 5-ALA/PpIX and ABY-029 (a conjugate of IRDye800CW and anti-EGFR Affibody molecules) in 12 rats with F98 EGFR-positive orthotopic glioma tumors. Dexamethasone resulted in a 42% decrease in the background signal of 5-ALA/PpIX, causing a 1.5x increase in contrast ($p < 0.01$). Interestingly, dexamethasone had no effect on the background signal of ABY-029, but resulted in a decrease in tumor uptake, causing a 2x reduction in contrast ($p < 0.005$). The spatial distribution of the dyes was also profoundly influenced by dexamethasone, which caused an accumulation of 5-ALA/PpIX at invasive tumor margins but caused the ABY-029 signal to recede from the margin. These preliminary results show the significant influence that concurrent oncological therapy can have on FGR contrast and provide important experimental insight for future human clinical studies.

10049-46, Session PSun

Pre-clinical development and safety testing of GMP produced ABY-029, fluorescent anti-EGFR affibody, for use in surgical resection

Jason R. Gunn, Sally M. Hull, Kimberley S. Samkoe, Kayla A. Marra, Thayer School of Engineering at Dartmouth (United States); Theresa V. Strong, The Univ. of Alabama at Birmingham (United States); P. Jack Hoopes D.V.M., Geisel School of Medicine, Dartmouth College (United States) and Univ. of Alabama (United States) and Thayer School of Engineering at Dartmouth (United States); Joachim

Feldwisch, Affibody AB (Sweden); Anders Wennborg, Affibody AB (Sweden) and Thayer School of Engineering at Dartmouth (United States); David W. Roberts, Geisel School of Medicine, Dartmouth College (United States); Keith D. Paulsen, Thayer School of Engineering at Dartmouth (United States) and Geisel School of Medicine, Dartmouth College (United States) and Univ. of Alabama (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States) and Univ. of Alabama (United States)

Anti-epidermal growth factor receptor (EGFR) affibody molecules have been developed for molecular targeting of EGFR with high affinity and uniquely designed to enable labeling with a single fluorescent molecule, or alternative imaging agents. Pre-clinical rodent data has strongly indicated that a near-infrared fluorescently labeled affibody molecule would make an excellent imaging agent for surgical guidance. A GMP production run of ABY-029, anti-EGFR affibody molecule (Z03115-Cys) conjugated to IRDye 800CW was recently finished, and tests of purity, potency, stability, biodistribution, pharmacokinetics toxicology and safety, and phototoxicity have demonstrated readiness for human use. The compound ABY-029 is a stable single site conjugate of IRDye® 800CW with EGFR binding affibody molecules. The drug product production was completed as expected based upon pre-GMP production testing, and filled into vials for human use. Toxicity testing on the pre-GMP compound was completed in Sprague Dawley rats at injection doses equivalent to 10X, 100X and 1000X human microdose levels (30 nmol microdose in human is equal to 24.5ug/kg in a rat). Pathology, Blood Chemistry and Hematology results showed no toxicity in either male or female rats after 24 hours and 2 weeks post injection. Real-time bio-specific interactions between ABY-029 and EGFR were performed using a Biacore x100 showing similar binding to both human (Kon= 8.338E5 M-1s-1, Koff= 1.069E-3 s-1, KD= 2.57E-9 M-1) and rat EFGR (Kon= 7.202E5 M-1s-1, Koff= 1.989E-2s-1, KD= 6.865E-10 M-1). Phase 0 open label, single-center trials are being planned for recurrent glioma, sarcoma and head and neck surgical resections.

10049-47, Session PSun

Review of fluorescence guided surgery systems: identification of key performance capabilities beyond ICG imaging

Alisha V. DSouza, Thayer School of Engineering at Dartmouth (United States); Huiyun Lin, Fujian Normal Univ. (China); Eric Henderson, Dartmouth Hitchcock Medical Ctr. (United States); Kimberley S. Samkoe, Geisel School of Medicine, Dartmouth College (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

There is growing interest in designing and using fluorescence imaging instruments to guide surgery based on signals related to tissue function and molecular phenotypes. While the clinical fluorescence guided surgery (FGS) field has been focused predominantly on indocyanine green (ICG) imaging, the accelerating development of new, more sophisticated molecular tracers should open potential indications for which FGS could present a paradigm change in how surgery is performed. There is a growing catalog of commercially marketed FGS systems, each with its own performance characteristics, and usage goals. A set of criteria to guide evaluation of a suitable instrument is proposed which encompasses: i) real-time overlay of white light and fluorescence images, ii) operation within ambient room lighting, iii) nanomolar level sensitivity, iv) quantitative capabilities, v) simultaneous multiple fluorophore imaging, and iv) ergonomic utility for open surgery. In this review, United States Food and Drug Administration (FDA) cleared commercial systems and some pre-market FGS research systems are evaluated to illustrate the continual increase in this performance feature base. Generally, the systems designed for ICG imaging only have a fraction of the desired features listed above, and lower sensitivity and dynamic range. In comparison, the emerging systems targeted for use with research agents have unique capabilities that can be essential for successful imaging studies with low concentration agents or with superior ambient light rejection performance. There is no perfect imaging system, but the feature differences between them are important differentiators in utility.

10049-48, Session PSun

Moving beyond single-point Raman spectroscopy: development of a hand-held Raman imaging probe for intraoperative tumor margin assessment

Karl St-Arnaud, Ecole Polytechnique de Montréal (Canada) and CRCHUM (Canada); Kelly Aubertin, CRCHUM (Canada) and Ecole Polytechnique de Montréal (Canada); Mathias Strupler, Ecole Polytechnique de Montréal (Canada); Michael Jermyn, Montreal Neurological Institute and Hospital (Canada) and Ecole Polytechnique de Montréal (Canada); Kevin Petrecca, Montreal Neurological Institute and Hospital (Canada); Dominique Trudel, CRCHUM (Canada); Frédéric Leblond, Ecole Polytechnique de Montréal (Canada) and Ctr. Hospitalier de l'Univ. de Montréal (Canada)

Tissue interrogation using Raman spectroscopy has been exploited to develop surgical guidance tools that can help improve the safety of tissue resection procedures as well as to minimize the volume of residual pathological tissue. This is especially important for applications involving microsurgical techniques where tissue information is typically required at sub-millimeter scales in critical areas at or close to margins between normal and pathological tissue. In the past, hand-held Raman probes have shown great potential to help surgeons discriminate between cancerous and normal tissue/cells. However, these tools are often limited to single-point analysis. Here, we present the development of a hand-held macroscopic Raman spectroscopy imaging probe with a field of view of 16mm? and a spatial resolution of 100μm and a working distance of 2 cm. The probe is composed of a coherent fiber optics imaging bundle with individual 10μm-diameter fibers as well as a lens/interference filters system insuring the Raman signal-to-noise ratio is maximized through optimal rejection of Rayleigh scattering associated with the 785 nm excitation light. The photo detection system consists in an imaging spectrometer coupled with a galvanometer for line scanning insuring hyperspectral Raman image can be acquired in <60s. Each pixel of the image forms a Raman spectrum covering the fingerprint region from 450cm-1 to 1750cm-1 with a spectral resolution of approximately 15cm-1. The hand-held probe was tested ex vivo on calf brain and human prostates. The ability of the system to recreate tissues maps of molecular content was validated using Neural Network classification.

10049-49, Session PSun

Towards real-time & quantitative endogenous properties imaging

Sylvain Gioux, Univ. de Strasbourg (France); Joseph P. Angelo, Boston Univ. (United States)

In this work we present our efforts towards building for the first time, a real-time, wide-field, quantitative imaging system for visualizing tissue properties. We base this system on our recent developments combining real-time acquisition with Single Snapshot of Optical Properties including 3D acquisition and correction (3D-SSOP) and real-time processing of information using novel ultra-fast lookup tables. Altogether, this work lays the foundation for the translation of endogenous optical imaging during surgical interventions.

10049-34, Session 9

Discovery of luminescence of water during radiation irradiation and application for medical physics *(Invited Paper)*

Seiichi Yamamoto, Nagoya Univ. School of Medicine (Japan)

Optical imaging detecting Cerenkov-light is a promising approach for molecular imaging or radiation therapy, but it was not yet conducted for proton therapy because light was not thought to be produced with the energy ranges because they are lower than Cerenkov-light threshold. Contrary to this consensus, our research group found that luminescence was emitted from water during proton-beam irradiation.

We placed water phantoms set on a table with a spot-scanning proton-therapy system, and luminescence images of water phantom were measured with a high-sensitivity cooled charge coupled device (CCD) camera during 100MeV proton-beam irradiation.

The luminescence images of water phantoms showed clear Bragg peak, and the measured proton ranges from the images were almost the same as those obtained with an ionization chamber. The image of the pure-water phantom also showed almost the same distribution as the tap-water phantom, indicating that the luminescence image was not related to impurities in the water.

We assume the luminescence was from the light emission from radicals produced in water by proton irradiations. If this hypothesis is true, other radiation irradiations to water will also produce the luminescence even though the energies are lower than Cerenkov-light threshold. Thus we tried the imaging of water for carbon-ion, alpha particles and low energy X-ray photons lower energy than Cerenkov-light threshold. The luminescence of water was observed and imaging was possible for all these radiations.

10049-35, Session 9

A dual-channel thoracoscope system for minimally invasive lung cancer surgery *(Invited Paper)*

Jie Tian, Institute of Automation (China)

In minimally invasive surgery, the white-light thoracoscope as a standard imaging tool is facing challenges of the low contrast between important anatomical or pathological regions and surrounding tissues. Currently, the near-infrared (NIR) fluorescence imaging shows superior advantages over the conventional white-light observation, which inspires researchers to develop novel imaging systems to improve overall outcomes of the endoscopic diagnosis. In this paper, we developed a NIR and white-light dual-channel thoracoscope system, which achieved high fluorescent signal acquisition efficiency with the simultaneous visualization of NIR and white-light dual channels. The system was design to have fast and accurate image fusion and high signal-to-background ratio (SBR) by optimizing both software algorithms and optical hardware components for better performance in the NIR spectrum band. We evaluated the system performances by measuring the minimally detectable concentration (0.305 ?g/ml) and spatial resolution (35 ?m). Furthermore, the feasibility of our system was verified by preclinical thoracoscopic experiments with indocyanine green in nine porcine models, and then a pilot clinical study with five lung cancer patients was conducted. The results demonstrated that our system has great potential for the future clinical applications.

10049-36, Session 9

Cherenkov-excited luminescence sheet imaging (CELSI) tomographic reconstruction

Jinchao Feng, Beijing Univ. of Technology (China) and Dartmouth College (United States); Petr Bruza, Dartmouth College (United States); Hamid Dehghani, The Univ. of Birmingham (United Kingdom); Scott C. Davis, Brian W. Pogue, Dartmouth College (United States)

As a new molecular imaging methodology, Cherenkov-excited luminescence sheet imaging (CELSI) utilizes thin sheets of radiation from a medical linear accelerator (LINAC) to produce a 2D sheet of Cherenkov photons within tissue, which in turn excites luminescence of molecular probes distributed in the volume. The precise knowledge of the light sheet position within the tissues allowed for accurate reconstruction of luminescent sources with high spatial resolution. In this work, a tomographic reconstruction algorithm for CELSI is proposed for the first time, to reconstruct distributions luminescence. Coupled continuous wave (CW) diffusion equations are used to model luminescent photon propagation in biological tissues. Since the light undergoes multiple scattering events before reaching the surface, the CELSI reconstruction is a severely ill-posed problem. Therefore, the CELSI reconstruction is achieved by minimizing the difference between measured and computed data, based on the Tikhonov regularization technique. The CELSI reconstruction was performed based on open source platform, NIRFAST. The feasibility and effectiveness of the reconstruction algorithm were tested with numerical simulations on noisy data. Contrast-detail analysis was then used to evaluate the imaging performance of CELSI reconstruction. Finally, comparisons of recovery luminescent sources using conventional diffuse optical fluorescence tomography (DOFT) and CELSI show that quantitative accuracy of recovered sources can be improved by the CELSI technique.

10049-37, Session 9

Cherenkov-excited imaging of molecular tracers in lymph nodes for estimation of receptor status

Alisha V. DSouza, Petr Bruza, Huiyun Lin, Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

Morbidity and complexity involved in lymph node staging via surgical resection and biopsy call for staging techniques that are less invasive. While visible blue dyes, fluorophores and lymphoscintigraphy are routinely used to locate the sentinel lymph nodes from the draining lymphatic vessels near a tumor, they rarely provide a metric to evaluate presence of cancer in those nodes in vivo. Using Lymph Node Molecular Concentration Imaging (LN-MCI) with a dual tracer approach in an in-vivo mouse metastasis model Tichauer et al.(1) demonstrated the ability to quantify micro-metastasis in nodes; here a fluorescent tracer targeted to cancer-specific cell receptors was injected and imaged along with a reference tracer to correct non-specific uptake and delivery heterogeneity. In order to allow for migration of such approaches to the clinical, there is a need for subsurface fluorescence imaging techniques adapted to perform dual fluorophore data acquisition.

Several studies have explored the ability to excite fluorophores using Cherenkov emission produced when high-energy radiation travels through tissue, such as during radiation therapy, using single fiber-probes(2) and tomographic arrangements(3), connected to spectrometers for detection. The Cherenkov light produced within tissue can be used to excite deep-seated fluorophores, such as within lymph nodes, and fluorescence emission is measured. As there is no scattering of the radiation beam, source excitation can be pro-duced deep within the tissue, down to ~3cm deep(4). Using spectrometers to measure the emitted signal, spectral contributions from multiple fluorophores can be relatively quantified to enable molecular

concentration imaging in lymph nodes. Various tissue-simulating phantoms and animal models were used to validate the approach and establish limits on spatial resolution, depth sensitivity, tracer concentration, and radiation dose.

Cherenkov molecular imaging is a novel imaging modality which has been demonstrated in vivo in a small animal model(5), and has strong diagnostic potential in imaging structural, metabolic, and immunologic probes for molecular imaging in both small and large animals. We anticipate that the low radiation dose, combined with the ability to image significantly deeper than conventional fluorescence imaging devices with high resolution (in theory ~0.1mm) Cherenkov molecular imaging will open new avenues and applications in the area of optical tracer usage for disease diagnosis and therapy monitoring.

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10049-38, Session 9

Cerenkov radiation-induced phototherapy for depth-independent cancer treatment

Walter J. Akers, Samuel Achilefu, Nalinikanth Kotagiri, Washington Univ. School of Medicine in St. Louis (United States)

Light emitted as the result of high-energy particle transport through biological tissues (Cerenkov radiation) can be exploited for noninvasive diagnostic imaging using high sensitivity scientific cameras. We have investigated the energy transfer potential of Cerenkov radiation, discovering a new phototherapeutic technique for treatment of localized and disseminated cancers. This technique, Cerenkov radiation-induced phototherapy (CRIT), like photodynamic therapy, requires the presence of both light and photosensitive agent together to induce cytotoxicity and effective cancer treatment. But unlike conventional phototherapy strategies in which tissue ablation or activation of photoactive molecules is limited to superficial structures, radiation-induced phototherapy enables phototherapy delivery to the tumor sites throughout the body. Titanium oxide nanoparticles, which produce cytotoxic reactive oxygen species upon irradiation with UV light, were targeted to tumor tissue by surface decoration with transferrin. Subsequent administration of tumor-avid radiotracer, 18-fluorodeoxyglucose (18FDG) provided localized UV light source via Cerenkov radiation. Treatment of tumor-bearing mice with the combination of Titanium nanoparticles and 18FDG resulted in effective reduction in tumor growth, while individual agents were not therapeutic. This new strategy in cancer therapy extends the reach of phototherapy beyond what was previously possible, with potential for treatment of cancer metastases and rescue from treatment resistance.

10049-39, Session 9

Fluorescence lifetime technique for detection of radiation-induced brain necrosis *(Invited Paper)*

Laura Marcu, Univ. of California, Davis (United States)

No Abstract Available

10049-40, Session 9

A tale of two photons: radioluminescence and its application in molecular imaging *(Invited Paper)*

Guillem Pratx, Stanford Univ. (United States)

Optical and ionizing radiation are two physical ways in which we can probe the living world. Until recently, these forms of radiation were used in distinct imaging and therapeutic applications—radiation therapy, photodynamic therapy, X-ray imaging, and diffuse optical tomography, to name a few. It has now been recognized that physical phenomena in which ionizing radiation and light are inherently coupled may provide powerful new capabilities for imaging and treating diseases. This presentation will review the physics and applications of radioluminescence, with a particular focus on molecular imaging. One such method, X-ray luminescence computed tomography (XLCT), uses narrow kilovolt X-ray beams to stimulate optical emissions from biologically targeted radioluminescent nanoparticles, thus providing high-resolution images even deep in tissue. A different phenomenon, Cherenkov luminescence, can also be harnessed to localize radiopharmaceuticals in vivo, allowing surgeons to visualize the molecular status of the tissues they are resecting. Recent progress towards routine implantation of these methods will be reviewed and sources of endogenous radioluminescence signal will be discussed.

Conference 10050: Clinical and Translational Neurophotonics

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10050-1, Session 1

Multi-scale spectrally-resolved fluorescence image guidance for brain tumour resection

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In glioma resection surgery, the detection of tumour is often guided by using intraoperative fluorescence imaging notably with 5-ALA-PpIX, providing fluorescent contrast between the normal brain tissue and the gliomas tissue. With the fluorescence image guidance, maximised tumour removal together with minimised damage of the healthy tissue are achieved, which translate to prolonged patient survival compared with the conventional white-light guided resection. However, the commercially available fluorescence imaging system relies on surgeon's eyes to visualise and distinguish the fluorescence signals, which makes the resection subjective and hinders patient outcomes. As such, there is a demand to develop spectrally-resolved quantitative fluorescence imaging systems to provide sufficient specificity and sensitivity on the fluorescence detection. Current research mainly focuses on developing fiber-based pointwise spectroscopy for quantitative assessment of 5-ALA-PpIX fluorescence. In this study, we present a novel multi-scale spectrally-resolved fluorescence imaging system and a computational model for reliably reconstruction of PpIX concentration in wide-field images. The system consists of a wide-field spectrally-resolved quantitative imaging device and a fluorescence endomicroscopic imaging system enabling in situ optical biopsy. The reconstruction model is based on the spectrally-resolved relative reflectance determined in both PpIX absorption and emission band. The image system and the model were evaluated with tissue-mimicking optical phantom experiments. Ex vivo human tumour sample studies demonstrated that the system was capable of specifically detecting the PpIX fluorescent signal in coregistered micro-scale and macro-scale, and estimation the true concentration of PpIX.

10050-2, Session 1

Optical tumor and blood vessel detection to enhance accuracy and safety of stereotactic brain tumor biopsies

Niklas Markwardt, Klinikum der Univ. München (Germany); Marcus H. Goetz, MRC Systems GmbH (Germany); Neda Haj-Hosseini, Univ. Hospital Linköping (Sweden); Ronald Sroka, Herbert Stepp, Laser-Forschungslabor, Ludwig-Maximilians-Univ. München (Germany); Petr Zelenkov, N.N. Burdenko Neurosurgical Institute (Russian Federation); Adrian Rühm, Laser-Forschungslabor, Ludwig-Maximilians-Univ. München (Germany)

A fiber-based mechano-optical device for stereotactic brain tumor biopsies is developed. 5-ALA-induced protoporphyrin IX (PpIX) fluorescence is used to localize vital tumor tissue; the spectral analysis of polychromatic light diffusely remitted from the investigated tissue serves to detect blood vessels and thus helps minimize the risk of cerebral hemorrhages.

For both tasks, ray tracing simulations and experiments on phantoms mimicking the optical properties of brain tumor tissue were performed. The sensitivity of PpIX-based tumor detection was investigated for two different excitation wavelengths (405 nm, 633 nm). The effect of blood interference was studied by placing artificial blood layers of 10-400 μm thickness between fiber and phantom. Blood vessel detection with a two-fiber probe (inter-fiber distance: 2 mm) was experimentally tested by using a blood vessel dummy, which was submerged into the tumor-mimicking phantom. The remitted light was analyzed at two wavelengths with strongly differing hemoglobin absorption (578 nm, 650 nm).

In general, 405-nm-excitation shows a 50-fold higher sensitivity, but physiological PpIX concentrations of a few μM should be well detectable with both wavelengths. In addition, 633-nm-excitation is considerably superior in case of blood-covered tumor tissue. For instance, a 50 μm blood layer blocks the 405-nm-excited fluorescence completely, but reduces the 633-nm-excited signal by less than 50%. Depending on their orientation, blood vessels are detectable up to 800-1200 μm ahead of the probe on the basis of a considerably reduced remission ratio I578 / I650 as compared to the background value.

The implementation into a conventional biopsy needle is proposed.

10050-3, Session 1

Optical guidance for stereotactic brain tumor biopsy procedures-preliminary clinical evaluation

Neda Haj-Hosseini, Johan Richter, Peter Milos, Martin Hallbeck, Karin Wårdell, Univ. Hospital Linköping (Sweden)

In the routine of stereotactic biopsy on suspected tumors located deep in the brain or patients with multiple lesions, tissue samples are harvested to determine the type of malignancy. Biopsies are taken from pre-calculated positions based on the preoperative radiologic images susceptible to brain shift. In such cases the biopsy procedure may need to be repeated leading to a longer operation time. To provide guidance for targeting diagnostic tumor tissue and to avoid vessel rupture on the insertion path of the tumor, an application specific fiber optic probe was developed. The setup incorporated spectroscopy for 5-aminolevulinic acid induced protopophyrin IX (PpIX) fluorescence in the tumor and laser Doppler for measuring microvascular blood flow which recorded backscattered light (TLI) at 780 nm and blood perfusion. The recorded signals were compared to the histopathologic diagnosis of the tissue samples (n=16) and to the preoperative radiologic images. All together 146 fluorescence and 276 laser Doppler signals were recorded along 5 trajectories in 4 patients. On all occasions strong PpIX fluorescence peaks were visible during real-time guidance. Comparing the gliotic tumor marginal zone with the tumor, the PpIX (51 vs. 528 a.u., [0-1790], $p < 0.05$) was higher and TLI (2.9 vs. 2.0 a.u., [0-4.1], $p < 0.05$) was lower in tumor. The autofluorescence (104 vs.70 a.u., [0-442], $p > 0.05$) and blood perfusion (8.3 vs. 17 a.u., [0-254], $p > 0.05$) were not significantly different. In conclusion, the optical guidance probe made real-time tumor detection and vessel tracking possible during the stereotactic biopsy procedures. Moreover, the fluorescence and blood perfusion in the tumor could be studied at controlled positions in the brain and the tumor.

10050-4, Session 1

Fiber-probe optical spectroscopy discriminates normal brain from focal cortical dysplasia in pediatric subjects

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Focal cortical dysplasia (FCD) is an abnormality in the cerebral cortex that is caused by malformations during cortical development. Currently, magnetic resonance imaging (MRI) and electro-corticography (ECoG) are used for detecting FCD. On the downside, MRI is very much insensitive to small malformations in the brain, while ECoG is an invasive and time consuming procedure. Recently, optical techniques were widely exploited as a minimally invasive and quantitative approaches for disease diagnosis. These techniques include fluorescence and Raman spectroscopy. The aim of this investigation is to study the diagnostic performances of optical spectroscopy incorporating fluorescence (at 378 nm and 445 nm excitation wavelengths) and Raman spectroscopy (at 785 nm excitation) for the discrimination of FCD from normal brain in pediatric subjects. The study included 10 normal and 17 FCD tissue sites from 2 normal and 8 FCD samples. The emission spectra of FCD at 378 nm excitation wavelength presented a blue-shifted peak with respect to normal tissue. Prominent spectral differences between normal and FCD tissue were observed at 880, 1002, 1265 and 1452 cm-1 using Raman spectroscopy. Tissue classification models were developed using multivariate statistical method, principal component analysis, in addition to an empirical algorithm based on a fluorescence spectral ratiometric approach. This study demonstrates that a combined spectroscopic approach can potentially provide a better diagnostic capability for classifying normal and FCD tissues. Further, the implementation of the technology within a fiber probe could open the way for in vivo diagnostics and intra-operative surgical guidance.

10050-5, Session 1

Differentiating functional brain regions using optical coherence tomography

Daniel A. Gil, Hansen C. Bow, Jin-H. Shen, Karen M. Joos M.D., Vanderbilt Univ. (United States); Melissa C. Skala, Morgridge Institute for Research (United States) and Univ. of Wisconsin–Madison (United States)

The human brain is made up of functional regions governing movement, sensation, language, and cognition. Unintentional injury during neurosurgery can result in significant neurological deficits and morbidity. The current standard for localizing function to brain tissue during surgery, intraoperative electrical stimulation or recording, significantly increases the risk, time, and cost of the procedure. There is a need for a fast, cost-effective, and high-resolution intraoperative technique that can avoid damage to functional brain regions. We propose that optical coherence tomography (OCT) can fill this niche by imaging differences in the cellular composition and organization of functional brain areas. We hypothesized this would manifest as differences in the attenuation coefficient measured using OCT. Five functional regions (prefrontal, somatosensory, auditory, visual, and cerebellum) were imaged in ex vivo porcine brains (n=3), a model chosen due to a similar white/gray matter ratio as human brains. The attenuation coefficient was calculated using a depth-resolved model and quantitatively validated with Intralipid phantoms across a physiological range of attenuation coefficients (absolute difference < 0.1cm-1). Image analysis was performed on the attenuation coefficient images to derive quantitative endpoints. We observed a statistically significant difference among the median attenuation coefficients of these five regions (one-way ANOVA, $p<0.05$). Nissl-stained histology will be used to validate our results and correlate OCT-measured attenuation coefficients to neuronal density. Additional development and validation of OCT algorithms to discriminate brain regions are planned to improve the safety and efficacy of neurosurgical procedures such as biopsy, electrode placement, and tissue resection.

10050-34, Session 1

In vivo clinical studies on brain tumor using time resolved fluorescence spectroscopy

Zhaojun Nie, Bartosz Bortnik, Fartash Vasefi, David Scott Kittle, Chirag G. Patil M.D., Ray M. Chu M.D., Adam N. Mamelak M.D., Keith L. Black M.D., Pramod V. Butte, Cedars-Sinai Medical Ctr. (United States)

Primary brain tumors, especially malignant gliomas, have a devastating effect on patients' lives. The only reliable method for intra-operative tissue diagnosis is the "frozen section". However, this time-consuming process can only be performed a limited number of times. Therefore, newer technologies are needed to aid the surgeon in achieving near-complete resection while avoiding damage to nearby eloquent areas. Time-resolved fluorescence spectroscopy (TRFS) measures dynamic fluorescence signals such as fluorescence lifetime in addition to fluorescence intensity. TRFS is particularly favored for in vivo diagnostic applications because fluorescence lifetime is independent of intensity variation artifacts common with in vivo measurements.

We have developed and employed such a system in the Operating Room (OR) to obtain in vivo measurements from patients undergoing elective brain tumor resection. All research was conducted under the approval of an Institutional Review Board (IRB). The intrinsic fluorescence decay features from different wavelength bands were extracted in real-time by using a fast data analysis method. A supervised learning algorithm using linear discriminant analysis (LDA) has been implemented to select features and to maximize statistical significance difference between training groups. The classification results were validated by histopathologic analysis and neuronavigation correlation to pre-operative MRI images. In this talk, we will present our latest normal cortex, white matter, and glioma results obtained from patient in vivo measurements.

10050-6, Session 2

Investigation of prefrontal cerebral hemodynamics during quantitative autonomic testing using NIRS

Zephaniah Phillips V, Seung-ho Paik, Korea Univ. (Korea, Republic of); Yoohwan Kim, Byung-Jo Kim, Korea Univ. Medical Ctr. (Korea, Republic of); Youngwoon Choi, Beop-Min Kim, Korea Univ. (Korea, Republic of)

In this work, we analyzed the clinical applicability of NIRS for use during Quantitative Autonomic Testing (QAT). QAT is a protocol consisting of deep breathing, Valsalva maneuver, and tilt table examination. It is used to diagnose a patient with disorders of the autonomic nervous system (ANS). Disorders of ANS includes orthostatic hyper/hypotension, vasovagal syncope, and postural orthostatic tachycardia syndrome. The results of QAT are typically analyzed with the use of blood pressure and heart rate data,

however these metrics may be influenced by factors such as arrhythmia, making the data interpretation and diagnosis difficult for clinicians. We tested our custom built 108-channel NIRS probe on 26 elderly patients during the QAT protocol with various ANS disorders. We found that prefrontal cerebral oxygenation correlated well with blood pressure and heart rate changes for all three tasks, making it a clinically feasible tool for observing ANS functionality. During the Valsalva maneuver, we observed a longer delayed and lower amplitude response of cerebral oxygenation to the prefrontal area in orthostatic intolerant patients. During the tilt table examination, we saw a larger response in cerebral oxygenation and less equal transient cerebral oxygenation during tilt up and tilt down in tilt table examinations that were positive (unhealthy), compared to tilt table examinations that were negative (healthy). Overall, our study showcases NIRS as an enhanced tool for understanding ANS disorders.

10050-7, Session 2

Functional connectivity during phonemic and semantic verbal fluency test: a multi-channel near infrared spectroscopy study

Chun-Jung Huang, Biomedical Optical Imaging Lab., National Chiao Tung Univ. (Taiwan); Chia-wei Sun, National Chiao Tung Univ. (Taiwan)

Verbal fluency tests (VFT) are widely used neuropsychological tests of frontal lobe and have been frequently used in various functional brain mapping studies. There are two versions of VFT based on the type of cue: the letter fluency task (LFT) and the category fluency task (CFT). However, the fundamental aspect of the brain connectivity across spatial regions of the fronto-temporal regions during the VFTs has not been elucidated to date. In this study we hypothesized that different cortical functional connectivity over bilateral fronto-temporal regions can be observed by means of multi-channel fNIRS in the LFT and the CFT respectively. Our results from fNIRS (ETG-4000) showed different patterns of brain functional connectivity consistent with these different cognitive requirements. We demonstrate more brain functional connectivity over left and right prefrontal region during LFT than CFT, and this was in line with previous brain activity studies using fNIRS demonstrating increased frontal and temporal region activation during LFT and CFT and more pronounced frontal activation by the LFT.

10050-8, Session 2

Prefrontal blood flow and oxygenation measured by NIRS during long-term memory tasks are impaired by acute hyperglycemia

R. Luke Harris, Lindsay Bell, Andrea Reimer, Jacqueline A. Pettersen, Paul Siakaluk, Brian Duffels, Univ. of Northern British Columbia Prince George Campus (Canada)

Our goal was to use 2-channel frequency domain near-infrared spectroscopy (NIRS) to investigate the hemodynamic and metabolic mechanisms underlying hyperglycemia-associated long-term memory impairment. We hypothesized that prefrontal cortex (PFC) oxygen saturation (%Sat) and perfusion (tHb, i.e. total hemoglobin) would decrease due to hyperglycemia during learning, and then increase during recall. During learning, participants' blood glucose was manipulated with beverages containing either 47.4 mg saccharine control (CON, n = 10), or 50 g dextrose + 23.7 mg saccharine (GLC, n = 10). In the Symbol-Digit Modalities Test (SMDT) participants matched nine symbols to corresponding digits (1-9 inclusive), completing 105 learning and 15 testing trials on day 1 and 15 testing trials on day 2. From learning to recall, CON SMDT performance was unchanged, but GLC SMDT performance was decreased 11% (P = 0.0173). There were significant interactions (2-way ANOVA) between the CON-GLC treatment effects and the learning-recall effects for both PFC perfusion and oxygen saturation. Specifically, comparing learning to recall, CON exhibited no tHb differences but for GLC there was a large tHb decrease during learning with a partial recovery toward CON values during recall (P = 0.0012); and, comparing learning to recall, CON exhibited a large %Sat decrease but GLC exhibited a large %Sat increase (P = 0.021). We speculate that, during learning, after overnight fasting (CON) the PFC demands more hemodynamic and metabolic resources and "works" harder, but with readily available sugar (GLC) the PFC exhibits decreased "effort."

10050-9, Session 2

Evaluation of mental focus signal based on near-infrared spectroscopy (NIRS)

Che Wei Chen, National Chiao Tung Univ. (Taiwan)

In this study, we evaluated the subjects' cognitive performance in a neuropsychological test, the Wisconsin card sorting test (WCST), by near-infrared spectroscopy (NIRS). Near-infrared spectroscopy is a noninvasive tool for measuring the hemodynamics in our brain. NIRS can emit two different wavelengths near-infrared light toward our tissue, then detecting the scattered light from the tissue. By means of the change between the incident and scattered light, NIRS can quantify the relative concentration in oxyhemoglobin and deoxyhemoglobin. Hemodynamics surrounding neurons is related to metabolic response in neuronal activity, so we can use the NIRS to access the neuronal activity in frontal lobe. The Wisconsin card sorting test has been commonly used in examining the lesions in frontal lobe. In many studies, the WCST also developed a clinical instrument to evaluate the change of cognitive function.

We classified subjects into two groups, good and bad performance in the WCST. According to the parameter in the WCST, good performers completed the whole task and bad performers fail in the task. Compare the hemodynamics of good and bad groups, we found the change of oxyhemoglobin concentration in good group is higher than bad group. According the result, we can conjecture the correlation between the degree of mental focus and the change of oxyhemoglobin in frontal cortex.

10050-10, Session 3

Coaxial cavity injected OCT and fiber laser ablation system for real-time monitoring of ablative processes, part 2: all fiber design and dispersion control

Jamil Jivraj, Jiaqi Zhou, Xijia J. Gu, Victor X. D. Yang M.D., Ryerson Univ. (Canada)

Inline optical coherence tomography (OCT) has proven to be an ideal feedback mechanism for real-time depth control of high-power ablation lasers. This has found use in industrial laser ablation applications, but it has the potential to truly change the use of laser ablation in medicine. Previously, we have presented a novel design that is able to place the OCT beam (?c = 1310nm) coaxially with the beam of a high-powered fiber laser (? = 1064nm, Pavg=10W, Ppeak = 1kW) without the need of a dichroic mirror on the output stage. This design successfully demonstrated real-time ablation depth feedback. Development of this design was continued and further refinements have been made to improve performance and form factor, with the ultimate goal being to create a compact, low-cost, high-precision laser scalpel to be used for various surgical osteotomies. We present an improved design that, unlike before, removes the need for bulk optics in the entire system other than a single collimator and doublet lens on the output. Strategies for dispersion mismatch compensation will be discussed to optimize resolution of OCT feedback. Initial results for depth-controlled ablation of tissue is presented.

10050-11, Session 3

The characterization of neural tissue ablation rate and corresponding peripheral heat damage of a 2 micron Tm doped fiber laser

Andrew J. Marques, Jamil Jivraj, Robnier Reyes, Joel Ramjist, Xijia J. Gu, Ryerson Univ. (Canada); Victor X. D. Yang M.D., Ryerson Univ. (Canada) and Sunnybrook Health Sciences Ctr., Univ. of Toronto (Canada) and Faculty of Medicine, Univ. of Toronto (Canada)

Tissue removal using electrocautery is standard practice in neurosurgery since tissue can be cut and cauterized simultaneously. Thermally mediated tissue ablation using lasers can potentially possess the same benefits but with increased precision. However, given the critical nature of the spine, brain, and nerves, the effects of direct photo-thermal interaction on neural tissue needs to be known, yielding not only high precision of tissue removal but also increased control of peripheral heat damage. The proposed use of lasers as a neurosurgical tool requires that a common ground is found between ablation rates and resulting peripheral heat damage.

Most surgical laser systems rely on the conversion of light energy into heat resulting in both desirable and undesirable thermal damage to the targeted tissue. Classifying the distribution of thermal energy in neural tissue, and thus characterizing the extent of undesirable thermal damage, can prove to be exceptionally challenging considering its highly inhomogenous composition when compared to other tissues such as muscle and bone. Here we present the characterization of neural tissue ablation rate and heat affected zone of a 1.94 micron thulium doped fiber laser for neural tissue ablation. In-Vivo ablation of porcine cerebral cortex is performed. Ablation volumes are studied in association with laser parameters. Histological samples are taken and examined to characterize the extent of peripheral heat damage.

10050-12, Session 3

In-situ photopolymerized and monitored spinal and brain implant

Andreas Schmocker, Azadeh Khoushabi, Pierre-Etienne Bourban, Dominique P. Pioletti, Christophe Moser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Photopolymerization is a common tool to harden materials initially in a liquid state. A surgeon can directly trigger the solidification of a dental implant or a bone or tissue filler. Traditionally, photopolymerization has been used mainly in dentistry. Over the last decade advances in material development including a wide range of biocompatible gel- and cement-systems open up a new avenue for in-situ photopolymerization.

We designed a miniaturized light probe where a photoactive material can be 1) mixed, pressurized and injected 2) photopolymerized or photoactivated and 3) monitored during the chemical reaction. The device enables surgeries to be conducted through a hole smaller than 500 ?m in diameter.

Using a combination of Raman and fluorescence spectroscopy, the current state of the photopolymerization was inferred and monitored in real time within an in-vitro tissue model. It was also possible to determine roughly the position of the probe within the tissue cavity by analysing the fluorescence signal. Using the technique hydrogels were successfully implanted into a bovine intervertebral disc model. Lately we used the device to implant a hydrogel into an aneurysm tissue cavity. The combination of an in-house-designed side-firing tip and the scattering hydrogel enabled a complete photopolymerization of the injected material. This results should pave the way for a new aneurysm treatment method.

10050-13, Session 3

Macrophages as drug delivery vehicles for photochemical internalization

Steen J. Madsen, Univ. of Nevada, Las Vegas (United States); Jonathan Gonzalez, Beckman Laser Institute and Medical Clinic, Univ. of California, Irvine (United States); Stephanie Molina, Univ. of Nevada, Las Vegas (United States); Rohit Kumar Nair, Henry Hirschberg M.D., Beckman Laser Institute and Medical Clinic, Univ. of California, Irvine (United States)

Targeted delivery of chemotherapeutic drugs to tumor sites is a major challenge in cancer chemotherapy. Cell-based vectorization of therapeutic agents has great potential for cancer therapy in that it can target and maintain an elevated concentration of therapeutic agents at the tumor site and prevent their spread into healthy tissue. The use of circulating cells such as monocytes/macrophages (Ma) offers several advantages compared to nanoparticles as targeted drug delivery vehicles. Ma can be easily obtained from the patient, loaded in vitro with drugs and reinjected into the blood stream. Ma can selectively cross the partially compromised blood-brain barrier surrounding brain tumors and are known to actively migrate to tumors, drawn by chemotactic factors, including hypoxic regions where conventional chemo and radiation therapy are least effective. The utility of Ma as targeted drug delivery vehicles for photochemical internalization (PCI) of tumors was investigated in this study.

In vitro studies were conducted using a mixture of F98 rat glioma cells and rat macrophages loaded with a variety of chemotherapeutic agents including bleomycin and 5-fluorouracil. Preliminary data show that macrophages are resistant to both chemotherapeutics while significant toxicity is observed for F98 cells exposed to both drugs. Co-incubation of F98 cells with loaded Ma results in significant F98 toxicity suggesting that Ma are releasing the drugs and, hence providing the rationale for their use as delivery vectors for cancer therapies such as PCI.

10050-14, Session 3

A comparison between antidepressant effects of transcranial near-infrared laser and citalopram in a rat model of depression

Farzad Salehpour, Neurosciences Research Ctr., Tabriz Univ. of Medical Sciences (Iran, Islamic Republic of); Seyed Hossein Rasta, School of Medical Sciences, Univ. of Aberdeen (United Kingdom) and Tabriz Univ. of Medical Sciences (Iran, Islamic Republic of); Gisou Mohaddes, Saeed Sadigh-Eteghad, Neurosciences Research Ctr., Tabriz Univ. of Medical Sciences (Iran, Islamic Republic of); Sima Salarirad, Tabriz Univ. of Medical Sciences (Iran, Islamic Republic of) and Univ. of Aberdeen (United Kingdom)

Introduction:

Depression is a common psychiatric disorder that its prevalence has been reported to be 16% among adults. In recent years, transcranial near-infrared laser therapy (NILT) has gained considerable attention as a novel non-pharmaceutical method for depression. The present study was designed to compare efficacy of two different treatment strategies in a rat model of depression.

Materials and Methods:

Forty male Wistar rats (180-200 g) divided in 4 groups: control, depressive, depressive-NILT and depressive-Citalopram. All animals excepted control group were exposed to chronic mild stress (CMS) for 4 weeks. Rats in laser

group received 10-Hz pulsed NILT (810 nm, energy density 1.2 J/cm2 per session) transcranially for a total of 12 sessions over a three-week period. Citalopram (10 mg/kg, Intraperitoneal) was administered for 21 consecutive days. Depressive-like behavior were tested in the forced swimming test (FST) model. Serum cortisol levels were also determined.

Results:

The results of FST showed an increase in swimming and decrease in immobility period, for both NILT and Citalopram groups compared to the stress group. There was also no significant difference between the experimental groups in climbing behavior. The induction of CMS significantly increased serum cortisol levels and treatments with NILT and Citalopram decreased it.

Conclusion:

Our findings showed that NILT will be more beneficial to improve the depressive-like behaviors in rat. Our data also showed that transcranial NILT was as effective as Citalopram in the treatment of depression. Therefore, these pieces of evidence may help improve NILT as an alternative non-pharmaceutical method for depression therapy.

10050-15, Session 4

Accuracy of neuro-navigated cranial screw placement using optical surface imaging

Raphael Jakubovic, Ryerson Univ. (Canada); Shuarya Gupta, Daipayan Guha, Todd Mainprize, Victor X. D. Yang M.D., Sunnybrook Health Sciences Ctr., Univ. of Toronto (Canada)

Cranial neurosurgical procedures are especially delicate considering that the surgeon must localize the subsurface anatomy with limited exposure and without the ability to see beyond the surface of the surgical field. Surgical accuracy is imperative as even minor surgical errors can cause major neurological deficits. Traditionally surgical precision was highly dependent on surgical skill. However, the introduction of intraoperative surgical navigation has shifted the paradigm to become the current standard of care for cranial neurosurgery.

Intra-operative image guided navigation systems are currently used to allow the surgeon to visualize the three-dimensional subsurface anatomy using pre-acquired computed tomography (CT) or magnetic resonance (MR) images. The patient anatomy is fused to the pre-acquired images using various registration techniques and surgical tools are typically localized using optical tracking methods. Although these techniques positively impact complication rates, surgical accuracy is limited by the accuracy of the navigation system and as such quantification of surgical error is required. While many different measures of registration accuracy have been presented true navigation accuracy can only be quantified post-operatively by comparing a ground truth landmark to the intra-operative visualization.

In this study we quantified the accuracy of cranial neurosurgical procedures using a novel optical surface imaging navigation system to visualize the three-dimensional anatomy of the surface anatomy. A tracked probe was placed on the screws of cranial fixation plates during surgery and the reported position of the centre of the screw was compared to the co-ordinates of the post-operative CT or MR images, thus quantifying cranial neurosurgical error.

10050-16, Session 4

Spinal intra-operative three-dimensional navigation with infra-red tool tracking: correlation between clinical and absolute engineering accuracy

Daipayan Guha, Univ. of Toronto (Canada); Raphael Jakubovic, Ryerson Univ. (Canada); Shaurya Gupta, Victor Yang, Univ. of Toronto (Canada)

Introduction:

Spinal intra-operative computer-assisted navigation (CAN) may guide pedicle screw placement. CAN techniques have been reported to reduce pedicle screw breach rates across all spinal levels. However, definitions of screw breach vary widely across studies, if reported at all. The absolute quantitative error of spinal navigation systems is theoretically a more precise and generalizable metric of navigation accuracy. It has also been computed variably, and reported in fewer than a quarter of clinical studies of CAN-guided pedicle screw accuracy.

Objective:

To characterize the correlation between clinical pedicle screw accuracy, based on post-operative imaging, and absolute quantitative navigation accuracy.

Methods:

We reviewed a prospectively-collected series of 209 pedicle screws placed with CAN guidance. Each screw was graded clinically by multiple independent raters using the Heary classification. Clinical grades were dichotomized per convention. The absolute accuracy of each screw was quantified by the translational and angular error in each of the axial and sagittal planes.

Results:

Acceptable screw accuracy, based on dichotomized Heary grade, was achieved for 95.1% of all screws, 93.4% of thoracic and 98.6% of lumbar screws. Inter-rater agreement was good for the Heary classification, significantly greater among radiologists than surgeon raters. Mean absolute translational/angular accuracies were 1.75mm/3.13 degrees and 1.20mm/3.64 degrees in the axial and sagittal planes, respectively. There was no correlation between clinical and absolute navigation accuracy.

Conclusions:

Radiographic classifications of pedicle screw accuracy vary in sensitivity across spinal levels, as well as in inter-rater reliability. Correlation between clinical screw grade and absolute navigation accuracy is poor, as surgeons appear to compensate for navigation registration error. Future studies of navigation accuracy should report absolute translational and angular errors. Clinical screw grades based on post-operative imaging may be more reliable if performed in multiple by radiologist raters.

10050-17, Session 4

3D Point cloud analysis of structured light registration in computer-assisted navigation in spinal surgeries

Shaurya Gupta, Daipayan Guha, Univ. of Toronto (Canada); Raphael Jakubovic, Ryerson Univ. (Canada); Victor X. D. Yang M.D., Ryerson Univ. (Canada) and Univ. of Toronto (Canada)

Computer-assisted navigation is used by surgeons in spine procedures to guide pedicle screws to improve placement accuracy and in some cases, to better visualize patient's underlying anatomy. Intraoperative registration is performed to establish a correlation between patient's anatomy and the pre/intra-operative image. Current algorithms rely on seeding points obtained directly from the exposed spinal surface to achieve clinically acceptable registration accuracy. Registration of these three dimensional surface point-clouds are prone to various systematic errors. The goal of this study was to evaluate the robustness of surgical navigation systems by looking at the relationship between the optical density of an acquired 3D point-cloud and the corresponding surgical navigation error. A retrospective review of a total of 48 registrations performed using an experimental structured light navigation system developed within our lab was conducted. For each registration, the number of points in the acquired point cloud was evaluated relative to whether the registration was acceptable, the corresponding system reported error and target registration error. It was

demonstrated that the number of points in the point cloud neither correlates with the acceptance/rejection of a registration or the system reported error. However, a negative correlation was observed between the number of the points in the point-cloud and the corresponding sagittal angular error. Thus, system reported total registration points and accuracy are insufficient to gauge the accuracy of a navigation system and the operating surgeon must verify and validate registration based on anatomical landmarks prior to commencing surgery.

10050-18, Session 4

Preliminary development of augmented reality systems for spinal surgery

Nhu Q. Nguyen, Joel M. Ramjist, Jamil Jivraj, Ho Yiu Cheng, Victor X. D. Yang, Ryerson Univ. (Canada)

Surgical navigation has recently been more actively deployed in open spinal surgeries due to the need for improved precision during procedures. This is increasingly difficult in minimally invasive surgeries due to the lack of visual cues on account of smaller exposure sites, and increases a surgeon's dependence on their knowledge of anatomical landmarks as well as the CT or MRI images.

The use of augmented reality (AR) systems and registration technologies in spinal surgeries could allow for improvements to techniques by overlaying a 3D reconstruction of patient anatomy in the surgeon's field of view, creating a mixed reality visualization. The AR system will be capable of projecting the 3D reconstruction onto a field and preliminary object tracking on a phantom. Dimensional accuracy of the mixed media will also be quantified to account for distortions in tracking. Cameras on the AR system are slightly offset compared to the user's vision and should be calibrated accordingly for increased accuracy.

10050-19, Session 4

Design of a head mounted optical tracking system for use in surgery

Ryan Deorajh, Peter Morcos, Jamil Jivraj, Joel M. Ramjist, Ryerson Univ. (Canada)

When using surgical loupes and other head mounted surgical instruments for an extended period of time, many surgeons experience fatigue during the procedure, which results in a lot of pain in the neck and upper back. This is primarily due to the surgeon being subjected to long periods of uncomfortable positions, due to the design of the surgical instrument. To combat this issue, the surgeon is required to have a larger freedom of movement, which will reduce the fatigue in the affected areas, and allow the surgeon to comfortably operate for longer periods of time.

The proposed design will incorporate an optical magnification system on a surgical head mounted display that will allow the surgeon to freely move their head and neck during the operation, while the optics are focused on the area of interest. The design will also include an infrared tracking system in order to acquire the field of view data, which will be used to control the optics. The reduction in neck pain will also be quantified using a clinically standardized numeric pain rating scale.

10050-20, Session 5

Assessment of hemodynamics of intracranial aneurysms using Doppler optical coherence tomography in patient specific phantoms: preliminary results

Joel M. Ramjist, Jamil Jivraj, Dexter Barrows, Ryerson Univ. (Canada); Barry Vuong, Massachusetts General Hospital (United States); Ronnie Wong, Victor X. D. Yang, Ryerson Univ. (Canada)

Intracranial aneurysms affect a large number of individuals every year. Changes to hemodynamics are thought to be a crucial factor in the initial formation and enlargement of intracranial aneurysms. Previously, surgical clipping – an open an invasive procedure, was the standard of care. More recently, minimally invasive, catheter based therapies, specifically stenting and coiling, has been employed for treatment as it is less invasive and poses fewer overall risks. However, these treatments can further alter hemodynamic patterns of patients, affecting efficacy and prognosis.

Doppler optical coherence tomography (DOCT) has shown to be useful for the evaluation of changes to hemodynamic patterns in various vascular pathologies, and intravascular DOCT may provide useful insight in the evaluation and changes to hemodynamic patterns before and during the treatment of aneurysms.

In this study, we present preliminary results of DOCT imaging used in three patient-specific aneurysm phantoms located within the Circle of Willis both pre and post-treatment. These results are compared with computational fluid dynamics (CFD) simulations and high-speed camera imaging for further interpretation and validation of results.

10050-21, Session 5

Multimodal optical imaging platform enables spatiotemporal mapping of cerebral blood flow and metabolism during cardiac arrest and resuscitation

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More than 500,000 people in the United States suffer cardiac arrest (CA) annually, and the majority of these patients face poor neurological outcome. A principal cause of this poor outcome is incomplete reperfusion of the brain following cardio-pulmonary resuscitation (CPR). Improvements in CA patient care may be enabled by detailed quantitative characterization of the relationship among cerebral perfusion, metabolism, and electroencephalogram (EEG) during the period immediately following CPR. To this end, we have developed a multi-modal platform consisting of EEG, laser speckle imaging (LSI), and multispectral spatial frequency domain (mSFDI) imaging. LSI provides two-dimensional maps of cerebral blood flow, and mSFDI obtains two-dimensional maps of concentrations of cerebral oxygenated and deoxygenated hemoglobin. In our study, LSI, mSFDI, and EEG data were acquired continuously from the brain in a rodent model of asphyxial CA and CPR. The measured cerebral blood flow and hemoglobin oxygenation can be employed to obtain spatio-temporal maps of the relative cerebral metabolic rate of oxygen consumption (rCMRO2), which can be inputted into a mathematical model to accurately predict the resumption of EEG activity ("EEG bursting"). Spatial mapping of blood flow and oxygenation reveals different temporal dynamics in large veins versus mixed arterio-venous regions. Collectively, this imaging platform is expected to enable quantitative characterization of neurovascular coupling in response to CA and CPR. The unique combination of this multi-modal platform and animal model is expected to facilitate rapid evaluation of

different clinically-translatable interventional techniques on the metabolic activity of the brain immediately following CPR.

10050-22, Session 5

In vivo imaging of tissue scattering parameter and cerebral hemodynamics in rat brain with a digital red-green-blue camera

Izumi Nishidate, Afrina Mustari, Tokyo Univ. of Agriculture and Technology (Japan); Satoko Kawauchi, Shunichi Sato, National Defense Medical College (Japan); Manabu Sato, Yamagata Univ. (Japan); Yasuaki Kokubo, Yamagata Univ. (Japan)

We propose a rapid imaging method to monitor the spatial distribution of total hemoglobin concentration (CHbT), the regional oxygen saturation (rSO2), and the scattering power b in the expression of musp=a(lambda)^-b as the scattering parameters in cerebral cortex using a digital red-green-blue camera. In the method, the RGB-values are converted into the tristimulus values in CIEXYZ color space which is a device-independent color system and compatible with the common RGB working spaces (NTSC, sRGB, etc). Monte Carlo simulation (MCS) for light transport in tissue is used to specify a relation among the tristimulus XYZ-values and the concentration of oxygenated hemoglobin (CHbO), that of deoxygenated hemoglobin (CHbR), and the scattering power b. In the present study, we performed sequential recordings of RGB images of in vivo exposed rat brain during the cortical spreading depression evoked by the topical application of KCl. Changes in the total hemoglobin concentration and the regional oxygen saturation imply the temporary change in cerebral blood flow during CSD. Triphasic change in the scattering power b was observed before the profound increase in the total hemoglobin concentration, which is indicative of the reversible morphological changes in brain tissue during CSD. The results in this study indicate potential of the method to evaluate the pathophysiological conditions in brain tissue with a digital red-green-blue camera.

10050-23, Session 5

Flow dynamics of cerebral penetrating arterioles has important implication to stroke penumbra development

Yuandong Li, Ruikang K. Wang, Univ. of Washington (United States)

Cerebral penetrating arterioles (PAs) are structurally and functionally different from the pial arterioles, as they are an exception group from the collateral circulation. Previous study has demonstrated the PAs are the bottlenecks to the flow from the surface arteries to the deeper microcirculations. However, functional change in PAs after ischemia plays an important role in delivering blood from a highly collateralized pial arteriole network to capillaries. An ability to separately monitor PA flow dynamics is critical to understand flow redistribution mechanism during stroke and refine stroke treatment target. We use optical coherence tomography (OCT)-based microangiography (OMAG) to evaluate flow and velocity change in multiple PAs after middle cerebral artery occlusion (MCAO) in mice across a large cortex region, covering distal branches of arterioles and anastomosis. We also apply OCT-based tissue injury mapping (TIM) method to reveal the potential penumbra development within the imaging region, upon which we observed apparent differences of the PA flow dynamics between core and penumbra regions. Our results suggest that the flow dynamics of PAs can be an important factor regulating the stroke penumbra development, and that stimulatory treatment targeting PAs can be studied under the guidance of OMAG.

10050-24, Session 5

Evaluation of spontaneous low-frequency oscillations in cerebral hemodynamics with time-series red-green-blue images

Izumi Nishidate, Afrina Mustari, Naoki Nakamura, Tokyo Univ. of Agriculture and Technology (Japan); Satoko Kawauchi, Shunichi Sato, National Defense Medical College (Japan); Manabu Sato, Yasuaki Kokubo, Yamagata Univ. (Japan)

The brain relies on a continuous and adequate supply of blood flow, bringing the nutrients that it needs and removing the waste products of metabolism. It is thus one of the most tightly regulated systems in the body, whereby a whole range of mechanisms act to maintain this supply, despite changes in blood pressure etc. Failure of these mechanisms is found in a number of devastating cerebral diseases, including stroke, vascular dementia and brain injury and trauma. Spontaneous contraction and relaxation of arterioles (and in some instances venules) termed vasomotion has been observed in an extensive variety of tissues and species. Vasomotion has a beneficial effect on tissue oxygenation and enhance blood flow. Although vasomotion is strictly a local phenomenon, the regulation of contractile activity of vascular smooth muscle cells is dependent on the complex interplay between vasodilator and vasoconstrictor stimuli from circulating hormones, neurotransmitters, endothelial derived factors, and blood pressure. Therefore, evaluation of the spontaneous oscillations in cerebral vasculatures might be a useful tool for assessing risk and investigating different treatment strategies in neurological disorders, such as traumatic brain injury, seizure, ischemia, and stroke. In the present study, we newly propose a method to visualize the spontaneous low-frequency oscillation of cerebral blood volume based on the sequential RGB images of exposed brain.

10050-25, Session 5

Alterations in cerebral metabolism observed in living rodents using fluorescence lifetime microscopy of intrinsic NADH

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Monitoring cerebral energy metabolism at a cellular level is essential to improve our understanding of healthy brain function and its pathological alterations. In this study, we resolve specific alterations in cerebral metabolism utilizing minimally-invasive 2-Photon fluorescence lifetime imaging (2P-FLIM) measurements of reduced nicotinamide adenine dinucleotide (NADH) fluorescence, collected in vivo from anesthetized rats and mice. Time-resolved lifetime measurements enables distinction of different components contributing to NADH autofluorescence. These components reportedly represent different enzyme-bound formulations of NADH. Our observations from this study confirm the hypothesis that NADH FLIM can identify specific alterations in cerebral metabolism. Using time-correlated single photon counting (TCSPC) equipment and a custom-built multimodal imaging system, 2-photon fluorescence lifetime imaging (FLIM) was performed in cerebral tissue with high spatial and temporal resolution. Multi-exponential fits for NADH fluorescence lifetimes indicate 4 distinct components, or 'species.' We observed distinct variations in the relative proportions of these components before and after pharmacological-induced impairments to several reactions involved in anaerobic glycolysis and aerobic oxidative metabolism. Classification models developed with experimental data correctly predict the metabolic impairments associated with bicuculline-induced focal seizures in separate experiments. Compared

to traditional intensity-based NADH measurements, lifetime imaging of NADH is less susceptible to the adverse effects of overlying blood vessels. Evaluating NADH measurements will ultimately lead to a deeper understanding of cerebral energetics and its pathology-related alterations. Such knowledge will likely aid development of therapeutic strategies for neurodegenerative diseases such as Alzheimer's Disease, Parkinson's disease, and stroke.

10050-26, Session 6

Diffuse optical systems and methods to image physiological changes of the brain in response to focal traumatic head injury

David Abookasis, Boris Volkov, Itamar Kofman, Ariel Univ. (Israel)

During the last four decades, various optical techniques have been proposed and intensively used for biomedical diagnosis and therapy both in animal model and in human. These techniques have several advantages over the traditional existing methods: simplicity in structure, low-cost, easy to handle, portable, can be used repeatedly over time near the patient bedside for continues monitoring, and offer high spatiotemporal resolution. In this work, we demonstrate the use of two optical imaging modalities namely, spatially modulated illumination and dual-wavelength laser speckle to image the changes in brain tissue chromophores, morphology, and metabolic before, during, and after the onset of focal traumatic brain injury in intact mouse head (n=15). Injury was applied in anesthetized mice by weight-drop apparatus using ~50gram metal rod striking the mouse's head. Following data analysis, we show a series of hemodynamic and structural changes over time including higher deoxyhemoglobin, reduction in oxygen saturation and blood flow, cell swelling, etc., in comparison with baseline measurements. In addition, to validate the monitoring of cerebral blood flow by the imaging system, measurements with laser Doppler flowmetry were also performed (n=5), which confirmed reduction in blood flow following injury. Overall, our result demonstrates the capability of diffuse optical modalities to monitor and map brain tissue optical and physiological properties following brain trauma.

10050-27, Session 6

Label-free quantitative molecular imaging of remyelination using Raman spectroscopy

Mads S. Bergholt, Andrea Serio, Imperial College London (United Kingdom); Amanda Boyd, Anna Williams, The Univ. of Edinburgh (United Kingdom); Molly M. Stevens, Imperial College London (United Kingdom)

Degeneration of myelin sheaths around axons plays a key role in the pathogenesis of multiple sclerosis (MS). The development of novel therapies heavily relies on our ability to quantify and understand the remyelination process. Immunofluorescence and electron microscopy remain the current gold standard for quantification of remyelination. Here, we present a comprehensive imaging approach based on correlative Raman spectroscopy and immunofluorescence for molecular imaging of remyelination. High quality Raman spectroscopic images were measured from cross-sections of brain tissue in an in vivo mouse model of focal demyelination induced with lysolecithin. Multivariate analysis of the Raman images and correlative myelin bound protein immunohistochemistry enabled quantification and molecular characterisation of remyelinating tissue at various time points. We develop quantitative metrics and show that remyelinated tissue is associated with a distinct molecular signature compared to control tissue. Finally we demonstrated the developed approach in MS lesions in human brain tissue. This work shows for the first time that Raman spectroscopy can be applied as novel tool to study remyelination in MS.

10050-28, Session 6

Fast diffuse correlation spectroscopy for non-invasive measurement of intracranial pressure

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Intracranial pressure (ICP) monitoring has a key role in the management of neurosurgical and neurological injuries. Currently, the standard clinical monitoring of ICP requires an invasive transducer into the parenchymal tissue or the brain ventricle, with possibility of complications such as hemorrhage and infection. A non-invasive method for measuring ICP, would be highly preferable, as it would allow clinicians to promptly monitor ICP during transport and allow for monitoring in a larger number of patients.

We have introduced diffuse correlation spectroscopy (DCS) as a non-invasive ICP monitor by fast measurement of pulsatile cerebral blood flow (CBF). The method is similar to Transcranial Doppler ultrasound (TCD), which derives ICP from the amplitude of the pulsatile cerebral blood flow velocity, with respect to the amplitude of the pulsatile arterial blood pressure. We believe DCS measurement is superior indicator of ICP than TCD estimation because DCS directly measures blood flow, not blood flow velocity, and the small cortical vessels measured by DCS are more susceptible to transmural pressure changes than the large vessels.

For fast DCS measurements to recover pulsatile CBF we have developed a custom high-power long-coherent laser and a strategy for delivering it to the tissue within ANSI standards. We have also developed a custom FPGA-based correlator board, which facilitates DCS data acquisitions at 50-100 Hz. We have tested the feasibility of measuring pulsatile CBF and deriving ICP in two challenging scenarios: humans and rats. SNR is low in human adults due to large optode distances. It is similarly low in rats because the fast heart rate in this setting requires a high repetition rate.

10050-29, Session 6

Optical coherence tomography of the cerebral cortex

Robnier Reyes Perez, Jamil Jivraj, Victor X. D. Yang M.D., Ryerson Univ. (Canada)

Spectral-Domain Optical Coherence Tomography (SD-OCT) provides a high speed, high resolution imaging technique with limited tissue depth penetration. The current use of OCT is limited to relatively small areas of tissue for anatomical structure diagnosis or minimally invasive guided surgery. In this study we propose to image the surface of the human cerebral cortex. This experiment aims to evaluate the potential difficulties encountered when applying OCT imaging to large and irregular surface areas.

The current state-of-the-art SD-OCT imaging technology uses scanning systems with at most 3 degrees of freedom (DOF) to obtain a 3D image representation of the sample tissue. We propose the use of a 7 DOF industrial robotic arm to increase the scanning capabilities of our OCT. In addition to having a flexible scanning platform, we use a high-speed InGaAs camera with a 1024 pixel resolution and 91,911 lines per second scanning speed. Such system will be capable of acquiring data from large samples of tissue that are too irregular for conventional methods. Advantages and disadvantages of our system are discussed in comparison to large area scanning techniques.

10050-30, Session 6

Graphics processor unit acceleration enables realtime endovascular Doppler optical coherence tomography imaging: development and validation

Dexter Barrows, Joel M. Ramjist, Ryerson Univ. (Canada); Barry Vuong, Harvard Medical School (United States); Kenneth K. C. Lee, 7D Surgical (Canada); Jamil Jivraj, Victor X. D. Yang M.D., Ryerson Univ. (Canada)

Endovascular Optical Coherence Tomography (OCT) has previously been used in both bench-top and clinical environments to produce vascular images, and can be helpful in characterizing, among other pathologies, plaque build-up and impedances to normal blood flow. The raw data produced can also be processed to yield high-resolution blood velocity information, but this computation is expensive and has previously only been available a posteriori using post-processing software. Real-time Doppler OCT (DOCT) imaging has been demonstrated before in the skin and eye, but this capability has not been available to vascular surgeons.

Graphics Processing Units (GPUs) can be used to dramatically accelerate this type of distributed computation. In this paper we present a software package capable of real-time DOCT processing and circular image display using GPU acceleration designed to operate with catheter-based clinical OCT systems. This image data is overlayed onto structural images providing clinicians with live, high-resolution blood velocity information to complement anatomical data.

Further, we validated flow data obtained in real time using a carotid flow phantom -- constructed using 3D structural OCT data -- and controlled flow from an external pump.

10050-31, Session PSun

In vivo optical properties of human brain tissue

Jaime J. Bravo, Jonathan D. Olson, Stephen C. Kanick, Thayer School of Engineering at Dartmouth (United States); David W. Roberts, Dartmouth Hitchcock Medical Ctr. (United States) and Geisel School of Medicine at Dartmouth (United States) and Norris Cotton Cancer Ctr., Dartmouth-Hitchcock Medical Ctr. (United States); Keith D. Paulsen, Thayer School of Engineering at Dartmouth (United States) and Geisel School of Medicine at Dartmouth (United States) and Norris Cotton Cancer Ctr., Dartmouth-Hitchcock Medical Ctr. (United States)

Tissue optical properties are known to play a major role in light-based diagnostic and therapeutic applications; however, a priori knowledge of optical properties of interest is still an avid area of research. In vivo estimates are valuable since stark differences have been found between optical properties measured in animal models or ex vivo human tissue compared to those measured in humans in vivo. There is a scarcity of in vivo data, understandably, when it comes to human brain tissue, with those summarizing the literature still tending to mix in vivo and ex vivo data, often with large variance in patient age and analysis methods, to obtain sufficient data for group analyses. As part of a clinical trial using 5-aminolevulinic acid (ALA) induced protoporphyrin IX (PpIX) for fluorescence guided surgery (FGS), we have been able to collect paired reflectance and fluorescence measurements of brain tissue for over 60 patients. Optical properties of sampled brain tissue were estimated from reflectance measurements using a monte-carlo-based look-up-table. The results serve, primarily, as an atlas of optical properties encountered in vivo over a range of brain tissues and tumor histologies. Apart from helping to account for optical property-based fluorescence distortions, for the first time, to the author's knowledge, we can compare the optical properties of normal and malignant adult brain tissue in vivo for a large group of patients determined using a common method.

10050-32, Session PSun

Development of zebrafish models of ischemic and hemorrhagic stroke using photochemical thrombosis and femtosecond-laser ablation

I-Ju Lee, National Chiao Tung Univ. (Taiwan); Yung-Jen Chuang, National Tsing Hua Univ. (Taiwan); Wei-Tien Chang, National Taiwan Univ. Hospital (Taiwan); Ian Liau, National Chiao Tung Univ. (Taiwan)

Stroke is a leading cause of adult death and disability and has remained a critical disease that attracts much clinical and translational research. Conventional stroke models require sophisticated surgery, hence suffering from unsatisfactory reproducibility. Zebrafish (Danio rerio) has recently emerged as a popular model organism because of varied attractive features; in particular, it is translucent at the larval stage, which greatly facilitates optical interrogation. Herein we report two laser-based methods to induce stroke in larval zebrafish. To mimic ischemic stroke, we selectively induced occlusion in target regions of the cerebral vasculature of zebrafish larvae that were injected with a photosensitizer (rose bengal) through photochemical means. Such photochemical thrombosis began with endothelial damage followed by vascular adhesion and aggregation of blood cells; the thrombus comprised thrombocytes and fibrins and was readily lyzed by injecting a thrombolytic agent (tissue plasminogen activator) to the blood stream; these observations conform to the characteristics of thrombosis/thrombolysis in humans. To mimic hemorrhagic stroke, we demonstrated the induction of extravascular breeding on larval zebrafish using femtosecond-laser ablation. Distinct degeneration and regeneration of the cerebral vasculature after hemorrhage were identified. The mortality and neurological outcome of zebrafish that had been induced ischemic or hemorrhagic stroke at varied regions of cerebral vasculature were evaluated. We anticipate our approach will not only improve our understanding of the pathophysiology but also facilitate the development of therapeutic interventions targeting stroke.

10050-33, Session PSun

Multimodal optical coherence tomography for in vivo imaging of brain tissue structure and microvascular network at glioblastoma

Konstantin Yashin, Privolzhsky Federal Research Ctr. (Russian Federation); Elena B. Kiseleva, Ekaterina V. Gubarkova, Nizhny Novgorod State Medical Academy (Russian Federation); Lev A. Matveev, Federal Research Ctr. Institute of Applied Physics of the Russian Academy of Sciences (Russian Federation); Maria M. Karabut, Vadim V. Elagin, Marina A. Sirotkina, Nizhny Novgorod State Medical Academy (Russian Federation); Igor A. Medyanik, Leonid Y. Kravets, Privolzhsky Federal Research Ctr. (Russian Federation); Natalia D. Gladkova, Nizhny Novgorod State Medical Academy (Russian Federation)

The aim of the investigation was to evaluate the performance of multimodal OCT (MM OCT) for differential diagnostics of normal brain and tumor tissue using an experimental model of glioblastoma. The spectral domain MM OCT device developed at the Institute of Applied Physics (Nizhny Novgorod, Russia) was used for the study. It provides two modes of investigation: cross-polarization OCT (CP OCT) and microangiographic OCT (MA OCT).

The OCT investigation was performed on an experimental 101.8 rat brain glioblastoma model. To evaluate the signal parameters typical of the tumor and of normal brain tissue, CP OCT and MA OCT images were compared with histological specimens (stained by hematoxylin and eosin). Analysis of the MA OCT images was performed on the basis of comparison with the findings of ZOOM-microscopy.

The comparative evaluation of the signals from the glial tumor and from normal brain tissue in co- and cross-polarizations showed significant differences between them and certain correlation with histopathology data. Furthermore we discovered that the brain under normal and pathological conditions have particular signal characteristics in cross-polarization that can give additional information about tissue structure. MA OCT allowed the visualization of the blood vessels both in the tumor and in the normal brain tissues, revealing changes in the form and sizes typical of the tumor vessels. We believe that multimodal OCT has great potential in visualization of different elements of brain tissue and further identification of clear features of glial tumors can allow to use MM OCT in intraoperative diagnosis.

10051-1, Session 1

Effect of hindpaw electrical stimulation on capillary flow heterogeneity and oxygen delivery in rodents in vivo *(Invited Paper)*

Yuandong Li, Wei Wei, Chenxi Li, Ruikang K. Wang, Univ. of Washington (United States)

We report a novel use of optical coherence tomography (OCT) based angiography to visualize and quantify dynamic response of cerebral capillary flow pattern in mice upon hindpaw electrical stimulation through the measurement of the capillary transit-time heterogeneity (CTH) and capillary mean transit time (MTT) in a wide dynamic range of a great number of vessels in vivo. The OCT system was developed to have a central wavelength of 1310 nm, a spatial resolution of ~8 μm and a system dynamic range of ~105 dB at an imaging rate of 92 kHz. The mapping of dynamic cerebral microcirculations was enabled by optical microangiography protocol. From the imaging results, the spatial homogenization of capillary velocity (decreased CTH) was observed in the region of interest (ROI) corresponding to the stimulation, along with an increase in the MTT in the ROI to maintain sufficient oxygen exchange within the brain tissue during functional activation. We validated the oxygen consumption due to an increase of the MTT through demonstrating an increase in the deoxygenated hemoglobin (HbR) during the stimulation by the use of laser speckle contrast imaging.

10051-2, Session 1

Visible light spectral domain optical coherence microscopy system for ex vivo imaging

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A spectral domain optical coherence microscope (OCM) was developed. A supercontinuum light source operating in the visible wavelength range of 465-690 nm was used. This extremely broad bandwidth enabled an ultrahigh axial resolution of 1.4 ?m in air (1.0 μm in tissue). The system consists of a Michelson interferometer and a homemade spectrometer. By using a CMOS camera with 8k pixels, an imaging depth of 2.4 mm (1.6 mm in tissue) and a roll-off of 11 dB/mm was achieved. Commercial objectives were used to focus the light onto the tissue. The system is operating in a scanning microscope configuration with a sample arm power of 970 ?W. Raster scanning of the tissue was performed by an integrated MEMS mirror providing a maximal field of view of 1x1 mm?. Imaging was performed at an A-scan rate of 30 kHz. Stained and unstained fragments of formalin-fixed, post-mortem brain tissue from human subjects were imaged. The results showed that various structures in the investigated brain tissue, such as white and gray matter, could be well distinguished based on their different scattering properties. Based on our preliminary data, visible light OCM imaging has the potential to provide 3D spectroscopic contrast of endogenous as well as of established immunohistochemical markers used in state-of-the-art neuropathological practice.

10051-3, Session 1

Imaging of functional hyperaemia using statistical parametric mapping and extended-focus optical coherence microscopy in the infrared spectral range

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Over the past decades, functional Magnetic Resonance Imaging has allowed us to monitor brain function through its ability to map cerebral function non-invasively over the entire brain by exploiting the different magnetic properties of oxygenated and deoxygenated blood. Alongside the advancements in fMRI technology, statistical tools have been devised in an effort to understand the neural pathways involved in sensory processing. Nevertheless, in spite of these major improvement, the achievable spatial and temporal resolution of fMRI limits its use as it is unable to resolve and monitor fast processes occurring at the cellular level. In this regard, Optical Coherence Microscopy is ideally suited as it can provide measurements of several hemodynamic parameters, including blood flow velocity, at a high temporal and spatial resolution. By shifting the central wavelength to the IR range and by engineering an extended-focus, we designed an OCM system capable of measuring quantitative blood flow velocity in deep cortical layers at high spatial resolution. The ability to measure quantitatively total blood velocity, as opposed to standard axial velocity Doppler OCM, is of paramount importance in brain imaging as a large portion of cortical vasculature is oriented perpendicularly to the optical axis. In an effort to analyze and characterize cortical hemodynamic reactivity, we devised a statistical framework by combining statistical parametric mapping tools, typically used in functional Magnetic Resonance Imaging, with the fast label-free three-dimensional imaging capabilities of extended-focus Optical Coherence Microscopy.

10051-4, Session 1

Assessing cortical and subcortical changes in a western diet mouse model using spectral / Fourier domain OCT

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The Western diet, causative in the development of atherosclerotic cardiovascular disease, has recently been associated with the development of diffuse white matter disease (WMD) and other subcortical changes. Yet, little is known about the pathophysiological mechanisms by which a high-fat diet can cause WMD. Mechanistic studies of deep brain regions in mice have been challenging due to a lack of non-invasive, high-resolution, and deep imaging technologies. Here we used Optical Coherence Tomography to study mouse cortical/subcortical structures noninvasively and in vivo. To better understand the role of Western Diet in the development of WMD, intensity and Doppler flow OCT images, obtained using a 1300 nm spectral / Fourier domain OCT system, were used to observe the structural and functional alterations in the cortex and corpus callosum of Western Diet and control diet mouse models. Specifically, we applied segmentation to the OCT images to identify the boundaries of the cortex/corpus callosum, and further quantify the layer thicknesses across animals between the two diet groups. Furthermore, microvasculature alterations such as changes in

spatiotemporal flow profiles within diving arterioles, arteriole diameter, and collateral tortuosity were analyzed. In the current study, while the arteriole vessel diameters between the two diet groups was comparable, we show that collateral tortuosity was significantly higher in the Western diet group, compared to control diet group, possibly indicating remodeling of brain vasculature due to dietary changes. Moreover, there is evidence showing that the corpus callosum is thinner in Western diet mice, indicative of tissue atrophy.

10051-5, Session 1

Imaging mouse cerebellum using serial optical coherence scanner

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We present the serial optical coherence scanner (SOCS), which consists of a polarization sensitive optical coherence tomography and a vibratome with associated controls for serial imaging, to visualize the cerebellum and adjacent brainstem of mouse. The cerebellar cortical layers and white matter are distinguished by using intrinsic optical contrasts. Images from serial scans reveal the large-scale anatomy in detail and map the nerve fiber pathways in the cerebellum and adjacent brainstem. The optical system, which has ~5.5 ?m axial resolution, utilizes a scan lens or a water-immersion microscope objective resulting in 10 ?m or 4 ?m lateral resolution, respectively. The large-scale brain imaging at high resolution requires an efficient way to collect large datasets. It is important to improve the SOCS system to deal with large-scale and large number of samples in a reasonable time. The imaging and slicing procedure for a section took about 4 minutes due to a low speed of the vibratome blade to maintain slicing quality. SOCS has potential to investigate pathological changes and monitor the effects of therapeutic drugs in cerebellar diseases such as spinocerebellar ataxia 1 (SCA1). The SCA1 is a neurodegenerative disease characterized by atrophy and eventual loss of Purkinje cells from the cerebellar cortex, and the optical contrasts provided by SOCS is being evaluated for biomarkers of the disease.

10051-6, Session 1

In-vivo imaging of the morphology and blood perfusion of brain tumours in rats with UHR-OCT

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Brain tumors are characterized with morphological changes at cellular level such as enlarged, non-spherical nuclei, microcalcifications, cysts, etc., and are highly vascularized. In this study, two research-grade optical coherence tomography (OCT) systems operating at ~800 nm and ~1060 nm with axial resolution of 0.95 μm and 3.5 μm in biological tissue respectively, were used to image in vivo and ex vivo the structure of brain tumours in rats. Female Fischer 344 rats were used for this study, which has received ethics clearance by the Animal Research Ethics Committees of the University of Waterloo and the University Health Network, Toronto. Brain tumours were induced by injection of rat brain cancer cell line (RG2 glioma) through a small craniotomy. Presence of brain tumours was verified by MRI imaging on day 7 post tumour cells injection. The in vivo OCT imaging session was conducted on day 14 of the study with the 1060 nm OCT system and both morphological OCT, Doppler OCT and OMAG images were acquired from the brain tumour and the surrounding healthy brain tissue. After completion of the imaging procedure, the brains were harvested, fixed in formalin and reimaged after 2 weeks with the 800 nm OCT system. The in vivo and ex vivo OCT morphological images were correlated with H&E histology. Results from this study demonstrate that UHR-OCT can distinguish between healthy and cancerous brain tissue based on differences in structural and vascular pattern.

10051-7, Session 2

Concurrent electrophysiology and TPM/OCT imaging of long-term implanted electrodes

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Microelectrodes implanted in the brain cause mechanical damage to the tissue that mediate neuroinflammation and eventual encapsulation by microglia and astrocytes. Electrophysiological signals recorded from implants used in brain-computer interfaces (BCI) degrade over time, limiting their usefulness, but the precise causes and progression are not fully understood. We are investigating the dynamics of brain morphological changes and neuroinflammation with a multimodal approach to better understand the potential causes of implant failure. We performed weekly optical coherence tomography (OCT)-guided two-photon microscopy (TPM) in the region around microelectrodes inserted under a cranial window concurrent with electrophysiological recordings. Transgenic mouse cohorts studied include Thy1-YFP, Cx3cr1, and GFAP-GFP to image neurons, microglia, and astrocytes, respectively. Single-shank, 16-channel, Michigan-style microelectrodes were inserted under the window at a 15-20° angle with an insertion depth up to cortical layer 5. Single-unit and local field potential (LFP) recordings were collected for 15 minutes while the animals moved freely in their home cages. Cellular and vascular morphology were monitored using TPM and OCT at timepoints matched to the recordings. In preliminary data, we observed a decay of neural firing rates in most of the channels after implantation. The relationship between electrophysiological measures (e.g., neural firing rate, LFP power) and neural/vascular morphological measurements (e.g., cell density, glial migration, blood flow changes) will be quantified. The multimodal approach combining electrophysiology and optical imaging provides a broader picture of the multifactorial nature of the response to implanted electrodes. Understanding and accounting for the response may lead to better BCI designs and approaches.

10051-8, Session 2

Optical changes in cortical tissue during seizure activity using optical coherence tomography

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Epilepsy is a chronic neurological disorder characterized by recurrent and unpredictable seizures. Electrophysiology has remained the gold standard of neural activity detection but its resolution and high susceptibility to noise and motion artifact limit its efficiency. Optical imaging techniques, including fMRI, intrinsic optical imaging, and diffuse optical imaging, have also been used to detect neural activity yet these techniques rely on the indirect measurement of changes in blood flow. A more direct optical imaging technique is optical coherence tomography (OCT), a label-free, high resolution, and minimally invasive imaging technique that can produce depth-resolved cross-sectional and 3D images. In this study, OCT was

used to detect non-vascular depth-dependent optical changes in cortical tissue during 4-aminopyridine (4-AP) induced seizure onset. Calculations of localized optical attenuation coefficient (μ) allow for the assessment of depth-resolved volumetric optical changes in seizure induced cortical tissue. By utilizing the depth-dependency of the attenuation coefficient, we demonstrate the ability to locate and remove the optical effects of vasculature within the upper regions of the cortex on the attenuation calculations of cortical tissue in vivo. The results of this study reveal a significant depth-dependent decrease in attenuation coefficient of nonvascular cortical tissue both ex vivo and in vivo. Regions exhibiting decreased attenuation coefficient show significant temporal correlation to regions of increased electrical activity during seizure onset and progression. This study allows for a more thorough and biologically relevant analysis of the optical signature of seizure activity in vivo using OCT.

10051-9, Session 2

Closed-loop optogenetic control of cortical hemodynamics

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The combination of optogenetics and optical imaging modalities has become a popular tool for the investigation of neurovascular coupling. Developing a closed-loop hemodynamic control system capable of dynamically following various blood flow patterns could be beneficial to the causal investigation of neurovascular coupling.

To develop this closed-loop hemodynamic control system, we have added a compensator to create a loop consisting of optogenetic stimulation, neural activities, neurovascular coupling, the evoked hemodynamic response, and a blood flow monitoring device to continuously minimize the difference between the recorded blood flow values and desired blood flow patterns.

A Real-time Doppler Optical Coherence Tomography (D-OCT) is employed in this study to monitor the cross-sectional velocity profile of a vessel at a frame rate of 71 frames per second. At the same time, a proportional-derivative compensator is used to continuously adjust the pulse width of a 450nm pulsed laser that delivers 15 Hz photostimulation to the cerebral cortex of Thy1-Channelrhodopsin-2 mice.

For each vessel, time-varying desired patterns and stimulation parameters were chosen according to the effect of pulse width modulation on its hemodynamic response, then proportional and derivative gains were tuned to produce a near-critically damped response.

After parameter optimization, the closed-loop hemodynamic compensator successfully controlled the blood flow in middle cerebral artery branches.

10051-10, Session 2

Spatial and temporal cerebral hemodynamic response after regional optogenetic stimulation in mice

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Coupling mechanisms between local neural activity and subsequent changes in cerebral hemodynamics are not fully understood. To conduct spatially and temporally precise cell type–specific neurovascular coupling studies, we have developed a real-time optogenetic-OCT platform that utilizes optogenetics, reinforced thinned skull surgical preparation, and high speed optical coherence tomography. This platform enables us to directly modulate neural activity of the cerebral cortex of ChR2 positive mice with desired spatial and temporal patterns, while monitoring the cross section of a blood vessel with a frame rate of 142 frames per second. We have used this platform to study the hemodynamic response to local optogenetic stimulation with varying irradiance, power, and stimulation spot size. For a spot size between 0.267mm and 0.8mm in diameter, the amplitude, rise-time, and fall time of blood flow and velocity responses were closely related to the stimulation optical power; however, no obvious relationship to the irradiance was observed under constant optical power. Arterial blood flow response to stimuli with various distances from the vascular territory was also investigated. The maximum blood flow response was found to happen at the center of the vascular territory. Stimulating neurons that are close to a vessel but further away from its territory does not evoke a perceptible vascular response.

10051-11, Session 3

Imaging human brain cytoarchitecture with quantitative optical coherence tomography *(Invited Paper)*

David A. Boas, Hui Wang, Ender Konukoglu, Bruce Fischl, Sava Sakadzic, Caroline V. Magnain, Athinoula A. Martinos Ctr. for Biomedical Imaging (United States)

No current imaging technology allows us to directly and without significant distortion visualize the microscopic and defining anatomical features of the human brain. Ex vivo histological techniques can yield exquisite planar images, but the cutting, mounting and staining that are required components of this type of imaging induce distortions that are different for each slice, introducing cross-slice differences that prohibit true 3D analysis. We are overcoming this issue by utilizing Optical Coherence Tomography (OCT) with the goal to image whole human brain cytoarchitectural and laminar properties with potentially 3.5 μm resolution in block-face without the need for exogenous staining. From the intrinsic scattering contrast of the brain tissue, OCT gives us images that are comparable to Nissl stains, but without the distortions introduced in standard histology as the OCT images are acquired from the block face prior to slicing and thus without the need for subsequent staining and mounting. We have shown that laminar and cytoarchitectural properties of the brain can be characterized with OCT just as well as with Nissl staining. We will present our recent advances to improve the axial resolution while maintaining contrast; improvements afforded by speckle reduction procedures; and efforts to obtain quantitative maps of the optical scattering coefficient, an intrinsic property of the tissue.

10051-12, Session 3

High-throughput dual-color precision imaging for brain-wide mapping of the connectome with cytoarchitectonic landmarks at the cellular level *(Invited Paper)*

Qingming Luo, Hui Gong, Jing Yuan, Xiangning Li, Anan Li, Tonghui Xu, Huazhong Univ. of Science and Technology (China)

Deciphering the fine morphology and precise location of neurons and neural circuits are crucial to enhance our understanding of brain function and diseases. Traditionally, we have to map brain images to coarse axial-sampling planar reference atlases to orient neural structures. However, this means might fail to orient neural projections at single-cell resolution due to position errors resulting from individual differences at the cellular level. Here, we present a high-throughput imaging method that can automatically obtain the fine morphologies and precise locations of both neurons and

circuits, employing wide-field large-volume tomography to acquire three-dimensional images of thick tissue and implementing real-time soma counterstaining to obtain cytoarchitectonic landmarks during the imaging process. The reconstruction and orientation of brain-wide neural circuits at single-neuron resolution can be accomplished for the same mouse brain without additional counterstains or image registration. Using our method, mouse brain imaging datasets of multiple type-specific neurons and circuits were successfully acquired, demonstrating the versatility. The results show that the simultaneous acquisition of labeled neural structures and cytoarchitecture reference at single-neuron resolution in the same brain greatly facilitates precise tracing of long-range projections and accurate locating of nuclei. Our method provides a novel and effective tool for application in studies on genetic dissection, brain function and the pathology of the nervous system.

10051-13, Session 3

A large field of view, high resolution Bessel beam two-photon light sheet microscope for large-scale 3D brain imaging

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Recent advances in light-sheet microscopy have made possible high-throughput large-scale imaging. The challenge remains however to achieve high-resolution imaging over very large areas because it is difficult to generate sufficiently thin and large light-sheets with existing technologies.

Here, we use a novel large field of view light-sheet microscope to obtain high-resolution whole brain 3D images in record time. A light-sheet is obtained by scanning a long thin near infrared Bessel beam into the sample while an sCMOS camera placed perpendicular to the scanning plane captures the emitted two-photon fluorescence. Using a scanned Bessel beam, the axial resolution remains constant over the entire field of view, yielding a high isotropic resolution.

Focusing a Gaussian beam with an axicon generates the Bessel beam. The length and the thickness of the beam are decoupled thereby achieving a large field of view (2.5mmx2.5mm) without compromising the axial resolution (2um).

Producing light-sheets with sufficient energy remains challenging. That is why we use of a regenerative-amplifier laser, which produces pulses with hundreds times more photons compared to conventional pulsed laser used in two-photon microscopy.

The new light-sheet microscope was successfully used to obtain a 3D map of dopaminergic neurons in clarified mice brains, with an axial resolution as high as 2um.

The combination of our light-sheet technology with optical clearing promises to transform our ability to understand the neuro-circuitry of the brain and thus significantly advance understanding of neurological and psychiatric diseases which involve remodelling of brain connections.

10051-14, Session 3

Bessel-beam light-sheet microscopy for high-fidelity functional and structural whole-brain imaging

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Light-sheet microscopy (LSM) has proven a useful tool in neuroscience and is particularly well suited to image the entire brain with high frame rates at single cell resolution. On the one hand, LSM is employed in combination with tissue clearing methods like CLARITY which allows for the reconstruction of neuronal or vascular anatomy over cm-sized samples. On the other hand, LSM has been paired with intrinsically transparent samples for real-time recording of neuronal activity with single cell resolution across the entire brain, using calcium indicators like GCaMP6.

Despite its intrinsic advantages in terms of high imaging speed and reduced photobleaching, LSM is very sensitive to residual opaque objects present in the sample, which cause dark horizontal stripes in the collected images. In the best case, these artefacts obscure the features of interest in structural imaging; in the worst case, dynamic shadowing introduced by red blood cells significantly alters the fluorescence signal variations related to neuronal activity.

We show how the use of Bessel beams in LSM can dramatically reduce such artefacts even in conventional one-sided illumination schemes, thanks to their “self-healing” properties. On the functional side, Bessel-beam LSM allows recording neuronal activity traces without any disturbing flickering caused by the movement of red blood cells. On the structural side, our proposed method is capable of obtaining anatomical information across the entire volume of whole mouse brains allowing tracing blood vessels and neuronal projections also in poorly cleared specimens.

10051-15, Session 4

Sub-micron opto-chemical probes for studying living neurons

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We have fabricated sub-micron opto-chemical probes with conformal functional coatings and demonstrated their application in intracellular and extracellular monitoring of neurons (cortical neuronal cultures and acute hippocampal slices). Using these probes, we have measured extracellular pH in the stratum radiatum in of the CA1 region of mouse hippocampus upon stimulation of presynaptic Schaffer collateral axons. Synaptic transmission was monitored using standard electrophysiological techniques. We find that the local pH transiently changes in response to synaptic stimulation. In addition, the geometry of the functionalized region on the probe combined with high sensitivity imaging enables simultaneous optical monitoring of spatially adjacent but distinct compartments. As proof of concept we impaled cultured neurons with the probe measured calcium and pH inside as well as directly outside of neurons as we changed the pH and calcium concentration in the physiological solution in the perfusion chamber. As such these probes can be used to study the impact of the environment on both cellular and extra-cellular space. Additionally as the chemical properties of the surrounding medium can be controlled and monitored

with high precision, these probes enable differential measurement of the target parameter referenced to a stable bath. This approach eliminates the uncertainties associated with non-chemical fluctuations in the fluorescent emission and result in a self-calibrated opto-chemical probe. We have also demonstrated multifunctional probes that are capable of measuring up to three parameters in the extracellular space in brain slices.

10051-16, Session 4

A novel reactive embedding resin for mapping of neural circuits

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Tissue embedding is a critical technology ensures image quality in biological tissue sectioning and imaging. The embedding resin largely affects the embedding effect in embedding process. However, in current plastic embedding technology, it is difficult to simultaneously satisfy both requirements of fluorescence preservation and serial section of large volume sample. Plastic embedding of large biological sample is particularly important for high-resolution, three-dimensional imaging. To improve the axial resolution of large-volume samples imaging, several well-established optical microscopy and electron microscopy imaging techniques have been combined with serial thin section technology. Therefore, plastic embedding resin should have sufficient mechanical performance to meet the requirement of thin serial-section. At present, the plastic embedding resins for biological tissues are mainly divided into two categories: epoxy resins and acrylate resins. Here, we developed a novel reactive plastic embedding resin to solve this problem. By crosslinking with the biological tissue via the reaction of epoxy group and amino group, this resin improves its compatibility with biological tissue. In the meantime, the embedding resin can be good to preserve the fluorescence intensity of endogenous fluorescent protein and dyes in biological tissue. This resin can potentially be applied in serial sectioning large sample for fluorescence microscopy imaging or electron microscopy imaging.

10051-17, Session 4

Hypertension and stroke: the changes in permeability of blood-brain barrier in rats

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It is well established that hypertension (HP) is main risk factor for stroke but mechanisms underlying this problem remain not clear. Indeed, many hypertensive patients have high blood pressure all life without stroke. What is crucial factor for hypertensive-related stroke? The stress is often reason contributing stroke in hypertensive subjects. Here we tested our hypnotize that high pressure exhausts tadaptive capacity of cerebral endothelium and blood-brain barrier (BBB) to stress, which can provokes stroke due to the increase in permeability of BBB and cytotoxic brain oedema.

Using model of renal HP in adult rats (n=37) we studied the permeability of BBB in early and chronic stages of disease. The dextran (70 kDa), fluorescent liposomes (100 nm), Evan Blue (confocal analysis) and gadolinium (MRI studies) did not permeate BBB in rats with masked and chronic stages of HP. Severe sound stress caused stroke only in animal group with chronic HP. In pre-stroke time, 85% of stressed hypertensive rats demonstrated the increase in BBB permeability to above indicated markers and these changes progressed in post-stroke period. Optical (laser speckle imaging, optical coherent tomography) and histological analysis showed HP formation is accompanied by ischemic changes in the brain but only stress induces brain oedema before and after stress-related stroke.

Thus, HP is accompanied by cerebral ischemia but without any changes in BBB permeability. The acute and strong stress causes the increase BBB permeability, which is associated with stroke in most hypertensive rats.

10051-18, Session 4

ReagentTF: A rapid and versatile optical clearing method for biological imaging

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The emergence of various optical clearing methods provides a great potential for imaging deep inside tissues by combining with multiple-labelling and microscopic imaging techniques. They were generally developed for specific imaging demand thus presented some non-negligible limitations such as long incubation time, tissue deformation, fluorescence quenching, incompatibility with immunostaining or lipophilic tracers. In this study, we developed a rapid and versatile clearing method, termed ReagentTF, for deep imaging of various fluorescent samples. This method can not only efficiently clear embryos, neonatal whole-brains and adult thick brain sections by simple immersion in aqueous mixtures with minimal volume change, but also can preserve fluorescence of various fluorescent proteins and simultaneously be compatible with immunostaining and lipophilic neuronal dyes. We demonstrate the effectiveness of this method in reconstructing the cell distributions of mouse hippocampus, visualizing the neural projection from CA1 (Cornu Ammonis 1) to HDB (nucleus of the horizontal limb of the diagonal band), and observing the growth of forelimb plexus in whole-mount embryos. These results suggest that ReagentTF is useful for large-volume imaging and will be an option for the deep imaging of biological tissues.

10051-19, Session 4

Subtle changes in myelination due to childhood experiences: label-free microscopy to infer nerve fibers morphology and myelination in brain

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Adverse childhood experiences have lasting detrimental effects on mental health and are strongly associated with impaired cognition and increased risk of developing psychopathologies. Preclinical and neuroimaging studies have suggested that traumatic events during brain development can affect cerebral myelination particularly in areas and tracts implicated in mood and emotion. Although current neuroimaging techniques are

quite powerful, they lack the resolution to infer myelin integrity at the cellular level. Recently demonstrated coherent Raman microscopy has accomplished cellular level imaging of myelin sheaths in the nervous system. However, a quantitative morphometric analysis of nerve fibers still remains a challenge. In particular, in brain, where fibres exhibit small diameters and varying local orientation. In this work, we developed an automated myelin identification and analysis method that is capable of providing a complete picture of axonal myelination and morphology in brain samples. This method performs three main procedures 1) detects molecular anisotropy of membrane phospholipids based on polarization resolved coherent Raman microscopy, 2) identifies regions of different molecular organization, 3) calculates morphometric features of myelinated axons (e.g. myelin thickness, g-ratio). We applied this method to monitor white matter areas from suicides adults that suffered from early live adversity and depression compared to depressed suicides adults and psychiatrically healthy controls. We demonstrate that our method allows for the rapid acquisition and automated analysis of neuronal networks morphology and myelination. This is especially useful for clinical and comparative studies, and may greatly enhance the understanding of processes underlying the neurobiological and psychopathological consequences of child abuse.

10051-40, Session PMon

Wearable ear EEG for brain interfacing and monitoring

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Brain-computer interfaces (BCIs) measuring electrical activity via electroencephalogram (EEG) have evolved beyond clinical applications to become wireless consumer products. Typically marketed for meditation and neurotherapy, these devices are limited in scope and currently too obtrusive to be a ubiquitous wearable. By leveraging the electrode positions inside the ear as established in previous research and grounding on the cartilage of the ear itself, the device could be comfortably worn in the ear all day and without any pinching of the ear lobe, enabling a new time-scale of consumer EEG data.

In this work, an ear-EEG device is created with a novel hybrid system that pairs hardware noise removal, selective software filtering, and post-processing for artifact removal and signal interpretation. A custom mobile app is implemented to process raw EEG from the device and display interpreted data to the user. Artifact removal and signal classification is accomplished via a combination of support matrix machines (SMMs) and soft thresholding of relevant statistical EEG properties.

Due to placement in the ear, this device eliminates artifacts of eye blinks, leaving just jaw clench to be filtered out. Additionally, the sensors are less sensitive to environmental noise due to the shielded RF environment provided by the ear. The small, compact, cost-effective, and discreet device is demonstrated against existing consumer electronics in this space for its high initial signal quality and resulting high post-processed signal to noise ratio (SNR), comfort, and usability.

10051-41, Session PMon

Online monitoring of tissue response to plasma coated rigid neural implants using fiber-based optical coherence tomography

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Microfabricated neuroprosthetic devices have made possible important observations on neuron activity; however, long-term high fidelity recording performance of these devices has yet to be realized. Tissue-device interactions appear to be a primary source of lost recording performance. The current state of the art for visualizing the tissue response surrounding brain implants in animals is Immunohistochemistry + Confocal Microscopy, which can only be performed after sacrificing the animal. Monitoring the tissue response as it develops could reveal important features of the response which may inform improvements in electrode design. Fiber-based Optical Coherence Tomography (OCT) has been adapted for imaging of brain tissue. Here, for the first time, we use OCT to achieve real-time, in-vivo monitoring of the tissue response surrounding chronically implanted rigid neural devices. The employed tissue response provoking implants are coated with plasma nanofilms, which has been demonstrated as a biocompatible and anti-inflammatory interface for indwelling devices. We evaluate the method by comparing the OCT results to traditional histology qualitatively and quantitatively. The differences in OCT signal across the implantation period between the plasma group and the control are also investigated.

10051-42, Session PMon

Functional near infrared spectroscopy for awake monkey to accelerate neurorehabilitation study

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Functional near-infrared spectroscopy (fNIRS) is suitable to measure brain functions during neurorehabilitation because of the portability and less motion restriction. However, it has not been well known whether the neural reconstruction can be observed through the changes in cerebral hemodynamics. In this study, we modified fNIRS system for measuring the motor function of awake monkeys to study the cerebral hemodynamics during neurorehabilitation.

The computer simulation was performed to determine optimal fNIRS source-detector interval for monkey motor cortex. Accurate digital phantoms (N=2) were constructed based on anatomical magnetic resonance images. The light propagation based on the diffusion equation was numerically calculated using the finite element method. The source-detector pair was placed on the scalp above the primary motor cortex. The interval conditions from 10 to 25 mm were examined. The results showed the detected intensity and partial optical path length in gray matter was decreased and increased by increase of source-detector interval, respectively. We found that 15 mm is the optimal interval for the fNIRS measurement of monkey motor cortex.

The preliminary measurement was performed on a healthy female macaque monkey using fNIRS equipment (OMM-3000, Shimadzu Corp., 32 channels) and custom-made optodes and their holder. The optodes were attached above bilateral primary motor cortices. Under the awaking condition, 10 to 20 trials of alternated single-sided hand movements for several seconds with intervals of 10 to 30 seconds were executed. Increases and decreases in oxy- and deoxy-hemoglobin were observed in the localized area in the hemisphere contralateral to the moved hand.

10051-43, Session PMon

Method for leveling the signal-to-noise ratio in multichannel functional near-infrared spectroscopy

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Functional near-infrared spectroscopy is potentially usable technique for monitoring the cortical functional hemodynamic change. Its so-called advantages such as the non-restraint measurement and the real-time activation monitoring, however, have not been successfully realized yet due to several technical drawbacks in fNIRS. Among these drawbacks, the difference in signal-to-noise ratio (SNR) within the measurement channels has been a serious problem, which makes difficult to compare the significance of the signal amplitude with baseline or those of other channels. The difference in SNR mainly originates from the difference in light loss due to the hair coverage etc. When two detectors receive different light intensities, the two detection signals are differently amplified through the calibration protocol in the commercial fNIRS equipments so as to effectively utilize dynamic range of the measurement. This protocol, however, differently amplifies the detection noise too, while their amplitudes are initially almost equal in both the detectors. Therefore, it makes different apparent noise and causes different SNR in each relevant channel. In order to level the SNRs in all the measurement channels, the system need to equalize the light intensities received by the detectors instead of conducting the conventional calibration. To realize this novel method for leveling the SNR in fNIRS channels, we developed an fNIRS system equipped with an optical attenuator at each source and detectors. A systematic procedure for modulating attenuators to level SNR over the channels was mathematically formulated, and the procedure was examined by using an optical phantom with partly hair-covered surface.

10051-44, Session PMon

A pH-sensitive red fluorescent protein compatible with hydrophobic resin embedding

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pH sensitive fluorescent proteins enabling chemical reactivation in resin are useful tools for fluorescence microimaging. EYFP or EGFP improved from GFP in jellyfish are good for such applications. For simultaneous two-color imaging, a suitable red fluorescent protein is of urgent need. Here a pH sensitive red fluorescent protein, pHuji, is selected and verified to be compatible with hydrophobic resin embedding and thus may be promising for dual-colour chemical reactivation imaging in conjuntion with EGFP or EYFP.

10051-45, Session PMon

Chemical sectioning: high throughput imaging brain networks ex vivo at synaptic resolution

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The complex anatomical structures of individual neurons and their synaptic connections form the signal transmission and processing pathway of the nervous system, and therefore are basis for understanding brain functions. However, existing imaging methods cannot cover the huge extension of neurons in mammalian brain with a resolution sufficient to identify connection sites (the pre- and postsynaptic structures). Here we proposed the chemical sectioning (CS) method that enables high throughput fluorescence imaging of a whole-mouse-brain at synaptic resolution. For the first time, we show the successful reconstruction of individual neurons extending axonal projections throughout the brain, including all dendrites, centimeter-extended axonal main stems, terminal arborizations, intensive dendritic spines and axonal boutons, and putative synapses. Our method enables quantitative analysis of the morphology, projections, and connectivity of genetically defined neurons. We found that three typical projections (the corticospinal, corticostriatal and corticothalamic projections) can be covered by one layer-V pyramidal neuron.

10051-46, Session PMon

Optical fiber based methods for deep brain calcium signal measurements in behaving mice

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Neural network activity is accompanied with dynamic transients of calcium ion concentration, which is visible for us by using calcium sensitive dyes or probes. Two-photon microscopy is a quite useful method to detect calcium signal of the superficial brain area of anesthetized or head fixed animals with perfect resolution and contrast. For deep brain calcium signal detection, optical fiber based approaches are usually used, and can be easily constructed for free moving animals. By using a single multi-mode fiber (MMF), calcium signal of a cluster of neurons could be detected with high temporal resolution. To simultaneously monitor neural activities in several brain areas of an animal or in different animals, we developed a multi-channel fiber photometry system. Using this system, we successfully recorded GCaMP6 fluorescent signals from the bilateral barrel cortices of a head-restrained mouse in a dual-channel mode, and the orbitofrontal cortices of multiple freely moving mice in a triple-channel mode. To detect the calcium signal of deep brain area with single cell resolution, we developed a laser scanning confocal microscope based on gradient index lens (GRIN lens). The frame rate of this system can reach 15 frames per second with 512?512 pixels in each frame, which is able to catch the dynamic process of calcium signal transients. A 500 ?m diameter GRIN lens can be easily implanted into a mouse deep brain with little injury, which will provide us a perfect 300 ?m diameter field of view with single cell resolution. This imaging system will be suitable for imaging deep brain area with a good temporal and spatial resolution?

10051-47, Session PMon

NeuroGPS-Tree: Automatic reconstruction of large-scale neuronal populations with dense neurites

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Mapping neuronal circuits is one of the central tasks in brain studies. For having a deep understanding in neural circuits, It is important to reconstruct neuronal populations. However, reconstruction of large-scale neuronal populations with dense neurites poses a challenge. Closely neurites contact, connect or overlap each other, which makes it difficult to identify falsely inferred links and separate neuronal tree in dense neuronal population. Most of existed software tools cannot identify individual neuronal trees in these neuronal population. Here we develop a novel method inspired by human strategies to separate individual neurons. This method can identify individual neuronal trees in dense neuronal population accurately and rapidly. For populations not resolvable by other methods, we obtained recall and precision rates of approximately 80%. We also demonstrate the reconstruction of 960 neurons within 3 h.

10051-48, Session PMon

A portable, multi-channel fNIRS system for prefrontal cortex: study on neurofeedback and imagery tasks

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Cerebral hemodynamic measurements can be thought as an indirect measurement of the brain's response to various stimuli. fNIRS is a neuroimaging technique which uses a near-infrared light source (in the range of 700-1000 nm) and allows for the detection of hemodynamic changes (i.e., oxygenated hemoglobin, deoxygenated hemoglobin, and total hemoglobin- which is proportional to blood volume). In this study, we developed a portable, multidistance prefrontal fNIRS system which has 12 light sources and 15 detectors for a total of 108 channels, with a sampling rate of 5 Hz. The wavelengths of the light source are 780nm and 850nm and are alternated sequentially. ATxmega128A1, an 8bit of Micro controller unit (MCU) with 200-4095 resolution, along with a MatLab interface was utilized for data acquisition.

Previously, we performed left and right arm motor imagery tasks that produced statistically significant changes of oxyhemoglobin concentration in the prefrontal cortex areas. We observed that the response localization for imagery tasks can be improved by carrying out neurofeedback training, during which the real-time hemodynamic response was given to the participating subject. In this study, we measured prefrontal and motor cortex oxyhemoglobin concentrations simultaneously during motor imagery task. We were able to detect a correlation of hemodynamic changes with the prefrontal and motor cortex. Furthermore we developed simple device for BMI (Brain Machine Interface) and tested it with neurofeedback training. Our portable fNIRS system and neurofeedback training may be useful in non-constraint environment for various clinical diagnoses and rehabilitation.

10051-49, Session PMon

High-throughput reconstruction of single neuronal morphology at brain-wide scale

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Reconstructing single neuronal morphology is an indispensable step in obtaining the structure of neuron circuit. Imaging the whole mouse brain at sub-micro resolution has provided a solid data base for this reconstruction. Reconstruct the single neuron at brain-wide scale remains challengeable. The challenges mainly lie in the generated dataset with the image size ranges from one to tens of terabytes, the neuronal fibers with discontinuous, tortuous and irregular morphologies. Most of the traditional reconstruction methods focus on specific problems, which are not general in solving the above challenges.

Here, we develop a self-learning reconstruction method, named Smart Tracer, which lowers the dependent upon threshold rules and reconstructs the complex neuronal morphologies throughout the brain. We adopt Support Vector Machine (SVM) to analyze the image features extracted from the images and build a decision function, determining whether the voxel is a foreground or not. In addition, the subsequent tracing procedure is accomplished by using mean-shift. We use Smart Tracer to test on various of datasets from multiple brain regions, the average accuracy is over 95%.

Furthermore, we developed a processing platform which breaks the bottleneck of mess data reading and management, which can rapidly read and edit any sub-volume from the whole brain dataset. We integrate the Smart tracer into the platform, named Smart Brain-wide Tracer. By using it, we achieve the high-throughput reconstruction of single neuronal morphology at brain-wide scale with little human interference. Compared with the manual reconstruction result, the tracing accuracy is nearly equal and the reconstruction speed is 10 times faster.

10051-51, Session PMon

Investigation soluble epoxide hydrolase inhibitor effects on cerebrovascular functions of focal ischemic stroke rats by novel dual-wavelength optical imaging

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Ischemic stroke is a high prevalence and lethal cardiovascular disorder caused by blood vessel blockage and leads to irreversibly brain damages and neurological dysfunctions, even death. The neural activity and which depends on local cerebral blood flow and oxygenation of hemoglobin was considered as neurovascular coupling for brain functions. Thus, the investigation of cerebrovascular dynamics plays an important role in elucidating the neurovascular functions and brain activity. However, the emerged optical imaging techniques for monitoring cerebrovascular dynamics remain with limitations, such as spatial and temporal resolutions. In addition, the current therapeutic strategies for ischemic stroke are mainly focused on blood redistribution of the ischemic penumbra by treating with tissue plasminogen activator (tPA) immediately. In the present study, we developed a full-field, high spatial resolution, fine temporal resolution and low costs dual-wavelength optical imaging system combined laser speckle contrast imaging (LSCI) with intrinsic optical signals imaging (IOSI), and the imaging system provides simultaneous recording of cerebral blood flow (CBF) and hemoglobin oxygenation through a cranial optical window. The effects of soluble epoxide hydrolase (sEH) inhibitor, 12-(3-adamantan-1-yl-ureido) dodecanoic acid (AUDA), on focal photothrombotic ischemia (PTI) of rats are also examined by measuring the dynamics of CBF and hemoglobin oxygenation by the dual-wavelength optical imaging system. The results demonstrate that AUDA not only significantly restored the CBF and oxygen-saturation level of hemoglobin, but also reduced the PTI stroke-induced brain damages. The optical imaging system provides a powerful diagnostic tool with full-filed high spatial and high temporal resolutions for cerebrovascular imaging.

10051-52, Session PMon

Polarization sensitive micro optical coherence tomography for cerebral cortex imaging

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Massachusetts General Hospital (United States); Xinyu Liu Sr., Linbo Liu, Nanyang Technological Univ. (Singapore)

Micro-OCT (?OCT) has been demonstrated being capable of delineating microstructures with 1-2 μm spatial resolution. However, the contrast of ?OCT is given by the sample refractive index difference, providing only anatomic information of tissue, which is lack of specificity in terms of tissue optical properties. In this presentation, we present a polarization sensitive ?OCT system (PS?OCT) with 2 ?m lateral resolution and 1 ?m axial resolution. By illuminating the sample with a circular polarized beam and detecting the two orthogonal polarization components in the backscattered light with two spectrometers, PS?OCT system can present additional contrasts, birefringence and optic axis orientation, to the reflectivity profile of tissue. Specimens of cerebral cortex from a mouse brain were imaged with the PS?OCT ex vivo. We found that the reflectivity and polarization changing of the cyto- and myelo-structures in the cerebral cortex of the mouse brain can be well identified. This system can provide a fast 3D preview of fresh, unprocessed and unstained brain sample, which may be of benefit to the research in brain science.

10051-53, Session PMon

Time-frequency functional brain connectivity during noxious stimulation by near infrared spectroscopy

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Functional near-infrared spectroscopy (fNIRS) is a neuroimaging technique that uses near-infrared light to obtain oxygenation and hemodynamic changes within the cerebral cortex. This technique has been applied in cortical activation detection and functional connectivity in brain research. Despite progress in functional interhemispheric research, most of the studies have been focused on the prefrontal cortex and little has been done to study the somatosensory region. In the present study, we use multichannel NIRS to assess the frequency-specific features of interhemispheric connectivity of somatosensory regions of the cortex after noxious stimulation. We recorded the hemodynamic activity in the somatosensory region (C3 and C4) of eleven healthy adults during an acupuncture stimulation procedure. Cross correlation was used to identify dominant channels in the region of interest. To clarify the coherence of functional connectivity, we calculated the wavelet transform coherence between corresponding channel pairs within the left and right somatosensory region. The results showed that functional connectivity between homologous channels in both hemispheres presented high coherence around three frequency ranges (0.3-0.15Hz, 0.15-0.075Hz, and 0.075-0.039Hz). Our results revealed that functional connectivity was significantly stronger during stimulation task and weaker during resting time. These findings suggest that synchronization of cortical activity in response to noxious stimulation reflects interhemispheric connectivity in the somatosensory region. This study contributes to the research field to investigate interhemispheric connectivity using NIRS and demonstrates the use of wavelet coherence transform to investigate functional connectivity.

10051-54, Session PMon

Resonance Raman spectroscopy of human brain metastasis of lung cancer analyzed by blind source separation

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Resonance Raman (RR) spectroscopy offers a novel optical biopsy method in cancer discrimination by a means of enhancement in Raman scattering. It is widely acknowledged that the RR spectrum of tissue is a superposition of spectra of various key building block molecules. In this study, the Resonance Raman (RR) spectra of human metastasis of lung cancerous and normal brain tissues excited by a selected wavelength at 532 nm are used to explore spectral changes caused by the tumor evolution. The diagnostic significance of RR spectra was investigated by blind source separation such as Principal Component Analysis (PCA). PCA is a statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components (PCs). The results show significant RR spectra difference between human metastasis of lung cancerous and normal brain tissues analyzed by PCA. To evaluate the efficacy of for cancer detection, a linear discriminant analysis (LDA) classifier is utilized to calculate the sensitivity, and specificity and the receiver operating characteristic (ROC) curves are used to evaluate the performance of this criterion. Excellent sensitivity of 0.97, specificity (close to 1.00) and the Area Under ROC Curve (AUC) of 0.99 values are achieved under best optimal circumstance. This research demonstrates that RR spectroscopy is effective for detecting changes of tissues due to the development of brain metastasis of lung cancer. RR spectroscopy analyzed by blind source separation may have potential to be a new armamentarium.

10051-55, Session PMon

Deception detection by hybrid-pair wireless fNIRS system

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Normal fNIRS setting up was limited by superficial physiological noises when applied into the lie detection. We designed a hybrid-pair wireless fNIRS system to improve the detection. The system takes advantages of short-pair channel to suppress the effect of physiological noises, and wireless module to improve the comfortableness of wearing it. We applied the system into a modified Guilty Knowledge Test. The experiment demonstrated that normal metrics might hint different energy consume during lying, while the regional oxygen saturation rSO_2, specific in the system, is sensitive to indicate a lying.

10051-20, Session 5

Rehabilitation-triggered cortical plasticity after stroke: in vivo imaging at multiple scales *(Invited Paper)*

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Neurorehabilitation protocols based on the use of robotic devices provide a highly repeatable therapy and have recently shown promising clinical results. Little is known about how rehabilitation molds the brain to promote motor recovery of the affected limb. We used a custom-made robotic platform that provides quantitative assessment of forelimb function in a retraction test. Complementary imaging techniques allowed us to access to the multiple facets of robotic rehabilitation-induced cortical plasticity after unilateral photothrombotic stroke in mice Primary Motor Cortex (Caudal Forelimb Area - CFA). First, we analyzed structural features of vasculature and dendritic reshaping in the peri-infarct area with two-photon fluorescence microscopy. Longitudinal analysis of dendritic branches and spines of pyramidal neurons suggests that robotic rehabilitation promotes the stabilization of peri-infarct cortical excitatory circuits, which is not accompanied by consistent vascular reorganization towards pre-stroke conditions. To investigate if this structural stabilization was linked to functional remapping, we performed mesoscale wide-field imaging on GCaMP6 mice while performing the motor task on the robotic platform. We revealed temporal and spatial features of the motor-triggered cortical activation, shining new light on rehabilitation-induced functional remapping of the ipsilesional cortex. Finally, by using an all-optical approach that combines optogenetic activation of the contralesional hemisphere and wide-field functional imaging of peri-infarct area, we dissected the effect of robotic rehabilitation on inter-hemispheric cortico-cortical connectivity.

10051-21, Session 5

Through-skull vasculature assessment using fluorescence brain imaging on murine models at around 800 nm

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Fluorescence brain imaging provides high spatial resolution and can serve as a complementary technique for computed tomography (CT) or magnetic resonance imaging (MRI). However, due to strong tissue scattering properties from scalp, cranium, dura matter and fluids, present fluorescence-based technologies requires invasive sample preparations such as craniotomy, cranial windows or skull-thinning procedures. To diminish this invasion, we propose and built a near-infrared fluorescence-based imaging setup for non-invasive brain imaging on live murine model with skull intact. The system uses a sCMOS camera operating around 800 nm with spatial resolution of 15.63 μm and obtains vasculature image with an average local SNR of 25 dB. The vasculature features are monitored via the inherent photoluminescence of the injected non-toxic indocyanine green fluorescence dye. An optimization of excitation power level in relation with the scattering observation and signal-to-noise ratio enhancement is performed. The study promotes future development of a non-invasive 3D monitoring of vasculature dynamic and neural circuit functions in the brain.

10051-22, Session 5

Optical projection tomography for the quantification of amyloid-beta plaques distribution in mouse models of Alzheimer's disease

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Optical projection tomography has emerged fifteen years ago as the analogue of X-ray computing tomography using visible light. It is able to image semi-transparent or cleared samples in the mesoscopic scale, i.e. at the centimeter level, with a resolution in the order of tens of micrometers. Compared to other techniques, OPT offers a simple optical solution making the setups affordable and compact, with the intrinsic advantages of optical imaging (i.e. specificity through fluorescent markers and enhanced resolution). Additionally, it benefits from all the advances in CT that can be translated to the optics regime. In this work, we introduce optical projection tomography to evaluate the distribution of amyloid-beta plaques in 5xFAD mouse brains, a widely used transgenic model of Alzheimer's disease. These plaques result from the aggregation of beta-amyloid fragments in the extra neuronal space and are a hallmark of the disease. We present a specifically designed instrument which can image the autofluorescence of the whole organ as well as an amyloid-binding dye, Methoxy-X04, specific to amyloid-beta plaques in the same brains. Furthermore, we compare two different clearing protocols: BABB (a clearing agent that matches the tissue refractive index) and X-CLARITY. We show that it is possible to image whole mouse brains with a resolution of approximately 30 micrometers using both clearing techniques, and that the quality of the images allows to further segment and quantify the plaques distribution across the different organ compartments. Ultimately, we show the development of the plaques over time and track their locations of appearance.

10051-23, Session 5

Simultaneous two-photon imaging of cerebral oxygenation and capillary blood flow in atherosclerotic mice

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Gradual changes in brain microvasculature and cerebral capillary blood flow occurring with atherosclerosis may significantly contribute to cognition decline due to their role in brain tissue oxygenation. This study aimed to investigate capillary blood flow and concomitant changes in brain tissue PO2 with atherosclerosis. Experiments were performed on young healthy C57Bl/6 mice (n=6, control) and atherosclerotic mice (n=6, ATX). Two-photon imaging of the left sensory-motor cortex during resting state under urethane (1.5 g/kg) anesthesia enabled us to measure both capillary dynamics and PO2. Animals received dextran-FITC injections through the tail vein and injections of the PO2 sensitive phosphorescent dye, PtP-C343, through the cranial window. Excitation was achieved by a MaiTai-BB laser operating at 840nm. The PtP-C343 probe and dextran-FITC probe were detected at 680 nm and 520 nm respectively, using two separate photomultiplier tubes (PMTs). We simultaneously measured oxygen tension and blood flow in capillaries using longitudinal and perpendicular scans of the vessels and oxygen tension in nearby tissue. Capillary diameter increased from 4.21±0.07 to 4.81±0.16 μm, RBC velocity increased from 0.66±0.04 to 1.05±0.09 mm/s and RBC flux increased from 46.5±3.88 to 49.77±5.95 cell/s, but hematocrit declined from 29.89±1.78 to 16.4±1.6 % with atherosclerosis. It was also observed that the adjoining PO2 values in capillaries increased from 35.94±1.48 to 38.87±1.13 mmHg with atherosclerosis and correlated with RBC flux (R2=0.36). The average tissue PO2 values around capillaries increased from 35.94±1.48 to 38.87±1.13 mmHg with atherosclerosis.

10051-24, Session 5

An optical assessment of the effects of glioma growth on resting state networks in mice

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Gliomas are known to cause significant changes in normal brain function that lead to cognitive deficits. Disruptions in resting state networks (RSNs) are thought to underlie these changes. However, investigating the effects of glioma growth on RSNs in humans is complicated by the heterogeneity in lesion size, type, and location across subjects. In this study, we evaluated the effects of tumor growth on RSNs over time in a controlled mouse model of glioma growth. Methods: Glioma cells (5x104-105 U87s) were stereotactically injected into the forepaw somatosensory cortex of adult nude mice (n=5). Disruptions in RSNs were evaluated weekly with functional connectivity optical intrinsic signal imaging (fcOIS). Tumor growth was monitored with MRI and weekly bioluminescence imaging (BLI). In order to characterize how tumor growth affected different RSNs over time, we calculated a number of functional connectivity (fc) metrics, including homotopic (bilateral) connectivity, spatial similarity, and node degree. Results: Deficits in fc initiate near the lesion, and over a period of several weeks, extend more globally. The reductions in spatial similarity were found to strongly correlate with the BLI signal indicating that increased tumor size is associated with increased RSN disruption. Conclusions: We have shown that fcOIS is capable of detecting alterations in mouse RSNs due to brain tumor growth. A better understanding of how RSN disruption contributes to the development of cognitive deficits in brain tumor patients may lead to better patient risk stratification and consequently improved cognitive outcomes.

10051-25, Session 5

Two-photon microscopy measurement of cerebral metabolic rate of oxygen using periarteriolar oxygen concentration gradients

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The cerebral metabolic rate of oxygen (CMRO2) is an essential parameter for evaluating brain function and pathophysiology. Measurements of CMRO2 with high spatio-temporal resolution are critically important for understanding how the brain copes with metabolic and blood perfusion changes associated with various clinical conditions, such as stroke, periinfarct depolarizations, and various microvasculopathies (e.g., Alzheimer's disease, chronic hypertension). CMRO2 measurements are also important for understanding the physiological underpinnings of functional Magnetic Resonance Imaging signals. However, the currently available approaches for quantifying CMRO2 rely on complex multimodal imaging and mathematical modeling. Here, we introduce a novel method that allows estimation of CMRO2 based on a single measurement modality - two-photon phosphorescence lifetime microscopy (2PLM) imaging of the partial pressure of oxygen (PO2) in cortical tissue. CMRO2 is estimated by fitting the changes of tissue PO2 around cortical penetrating arterioles with the Krogh cylinder model of oxygen diffusion. We measured the baseline CMRO2 in anesthetized rats, and modulated tissue PO2 levels by manipulating the depth of anesthesia. This method has a spatial resolution of approximately 200 ?m and it may provide CMRO2 measurements in individual cortical layers or within confined cortical regions such as in ischemic penumbra and the foci of functional activation.

10051-26, Session 6

A three-photon microscope with adaptive optics for deep-tissue in vivo structural and functional brain imaging

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Two-photon fluorescent microscopy has revolutionized neurobiological research as it enables observation of the structural and functional changes of neural circuits in the living brain with subcellular resolution. However, scattering and refractive aberration in the tissue limit direct, non-invasive access to brain structures more than a few hundred micrometers below the cortical surface, such as deep cortical layers and subcortical regions. Using a combination of three-photon excitation to reduce scattering with adaptive optics to correct for refractive aberrations, we imaged subcellular structures of deep-layer cortical neurons labeled by a red fluorescent protein in the living mouse over time. We also probed the functional dynamics of such neurons by imaging their calcium transients using a red-shifted, genetically encoded calcium indicator, while the animal is awake and behaving. These results demonstrate three-photon adaptive optics microscopy as a promising tool to explore the structural plasticity and functional organization of neural circuits in deep brain areas.

10051-27, Session 6

Measurement of cortical functional activation in awake mice using two-photon microscopy and a new PO2-sensitive probe

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We characterized cortical microvascular PO2 and blood flow changes in response to whisker stimulation in awake mice. The measurements were performed by combining two-photon microscopy imaging of the cortical oxygenation and optical coherence tomography imaging of the

cerebral blood flow. In order to perform fast spatio-temporally resolved measurements of PO2, we used a newly-developed oxygen-sensitive probe PtG-2P, which has significantly higher brightness than the established two-photon-enhanced oxygen sensor PtP-C343. We characterized the performance of the new probe in vivo and mapped the amplitudes and shapes (e.g. initial dip, overshoot, and post stimulus undershoot) of the PO2 changes as a function of the vessel type (e.g., arterioles, capillaries, and venules) and a distance from the activation center. The measurements in the awake mice are not affected by the confounding factors of anesthesia on the animal physiology, including the level of cerebral metabolism and the amplitude and speed of neuronal and vascular responses. Our results will help to understand changes in oxygenation and blood flow on the cortical microvascular scale, will lead to improved understanding of the cerebral physiology, pathophysiology and will improve quantitative interpretation of fMRI signals.

10051-28, Session 6

Rapid imaging of mammalian brain slices with a compact light sheet fluorescent microscope

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Light sheet fluorescent microscopy (LSFM) has been proven to provide high acquisition speed and high contrast image, as well as low photo-bleaching and photo-damage. These advantages make LSFM particularly suitable for repeated imaging of live tissues, for example in studies of neuronal development.

Here we describe a compact setup design. It features a 45° inverted geometry and an integrated photolysis laser, light sheet scanning mirror and electrical tunable lens on detection arm. This setup is optimized for applications in neuroscience, in particular fast imaging of sub-neuronal structures in mammalian brain slices.

We report the prototype instrument is capable of rapid imaging wide area (300 ? 300 ?m) of the dendritic or axonal arbor of a dye-filled neuron in hippocampal slice. We demonstrate several applications of this novel, compact LSFM, including: the acquisition of a 2-channel Z-stack image of a living, large pyramidal neuron filled with two different fluorescent dyes; a time series showing Ca2+ influx during photolytic stimulation of a single spine; a time series showing action potential-evoked Ca2+ influx into presynaptic terminals over a substantial length of axon; and a time series showing spontaneous neurotransmitter release evoking Ca2+ influx in dendritic spines. Thus we demonstrate that our approach to LSFM offers a powerful new functionality to the neuroscience community that is not achievable with traditional imaging methods.

10051-29, Session 6

Exploring infrared neural stimulation with multimodal nonlinear imaging

Wilson R. Adams, Anita Mahadevan-Jansen, Vanderbilt Univ. (United States)

Infrared neural stimulation (INS) provides optical control of neural excitability using near to mid-infrared (mid-IR) light, which allows for spatially selective, artifact-free excitation without the introduction of exogenous agents or genetic modification. Although neural excitability is mediated by a transient temperature increase due to water absorption of IR energy, the molecular nature of IR excitability in neural tissue remains unknown. Current research suggests that transient changes in local tissue temperature give rise to a myriad of cellular responses that have been individually attributed to IR mediated excitability. To further elucidate the underlying biophysical mechanisms, we have begun work towards employing a novel multimodal nonlinear imaging platform to probe the molecular underpinnings of INS. Our imaging system performs coherent anti-Stokes Raman scattering (CARS), stimulated Raman scattering (SRS), two-photon excitation fluorescence (TPEF), second-harmonic generation (SHG) and thermal imaging into a single platform that allows for unprecedented co-registration of thermal and biochemical information in real-time. Here, we present our work leveraging CARS and SRS in acute thalamocortical brain slice preparations. We observe the evolution of lipid and protein-specific Raman bands during INS and electrically evoked activity in real-time. Combined with two-photon fluorescence and second harmonic generation, we offer insight to cellular metabolism and membrane dynamics during INS. Thermal imaging allows for the coregistration of acquired biochemical information with temperature information. Our work previews the versatility and capabilities of coherent Raman imaging combined with multiphoton imaging to observe biophysical phenomena for neuroscience applications.

10051-30, Session 7

Photoacoustic microscopy of cerebral hemodynamic and metabolic responses to anesthetics

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It remains a challenge to directly assess general anesthetics-induced hemodynamic and oxygen-metabolic changes from the true baseline under wakefulness at the microscopic level, due to the lack of an enabling technology for high-resolution imaging of the awake rodent brain.

Here, we report head-restrained photoacoustic microscopy (PAM), which enables simultaneous imaging of the cerebrovascular anatomy, total concentration and oxygen saturation of hemoglobin (CHb and sO2), and blood flow in awake mice. Combining these hemodynamic measurements allows us to derive two key metabolic parameters—oxygen extraction fraction (OEF) and the cerebral metabolic rate of oxygen (CMRO2). Side-by-side PAM comparison of the awake and anesthetized rodent brains (N=5) revealed multifaceted cerebral responses to isoflurane, an anesthetic widely used in preclinical research and clinical practice. Briefly, following the exposure to 1.6% isoflurane, the venous sO2 increased from 65±4% to 86±6% in contrast to the unchanged arterial sO2. In addition, marked arterial dilation (from 34±4 μm to 44±4 μm) and moderate venous dilation (from 43±5 μm to 48±6 μm) were observed. Accompanying vasodilation was significant increase in the blood flow speed (on average, 54% in arteries and 37% in veins). Combining the multiple hemodynamic parameters, we revealed that the isoflurane induced a 73% decrease in OEF and a 50% decline in CMRO2. Extending the isoflurane study to other volatile and intravenous anesthetics is ongoing.

The head-restrained PAM opens a new avenue for research on neurovascular coupling without the influence of anesthesia and on the neuroprotective effects of various interventions against cerebral hypoxia and ischemia.

10051-31, Session 7

Real-time reperfusion imaging for cerebral ischemia in rats using the multi-wavelength handheld photoacoustic system

Yu-Hang Liu, Yu Xu, Kim Chuan Chan, Kalpesh Mehta, National Univ. of Singapore (Singapore); Nitish V. Thakor, National Univ. of Singapore (Singapore) and Johns Hopkins Univ. (United States); Lun-De Liao, National Univ. of Singapore (Taiwan) and Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes (Singapore)

Stroke is the second leading cause of death worldwide. Rapid and precise diagnosis is essential to expedite clinical decision and improve functional outcomes in stroke patients; therefore, real-time reperfusion imaging plays an important role to provide crucial information for post-stroke recovery analysis. In this study, based on the multi-wavelength laser and 18.5 MHz array-based ultrasound transducer, a real-time handheld photoacoustic (PA) system was developed to evaluate cerebrovascular functions pre- and post-stroke in rats. Using this system, hemodynamic information including cerebral blood volume (CBV) and hemoglobin oxygen saturation (SO2) can be acquired for assessment. Two rat stroke models (i.e., photothrombotic ischemia (PTI) and middle cerebral artery occlusion (MCAo)) were employed for evaluating the difference between local and global ischemia in reperfusion mechanism. For achieving better intrinsic PA contrast, COMSOL simulation was applied to optimize the light delivery (e.g., laser beam incident angle) from customized fiber bundle, while phantom experiment was conducted to evaluate the imaging resolution of this system. Results of phantom experiments show that hair (~70 μm diameter) and pencil lead (500 μm diameter) can be imaged clearly. On the other hand, results of in vivo experiments demonstrate that stroke symptoms like hypoxia can be observed in both PTI and MCAo models post-stroke. In the near future, with the help of PA specific contrast agent, the system will be able to achieve blood-brain barrier leakage imaging post-stroke. Overall, the real-time handheld PA system holds great potential in disease models involving impairments in cerebrovascular functions.

10051-32, Session 7

Photoacoustic imaging of biopotentials: a feasibility study

Nashaat S. Rasheed, John R. Cressman, Parag V. Chitnis, George Mason Univ. (United States)

Currently, the most researched noninvasive approach for monitoring neuro-electrical activity involves optical-fluorescence imaging, which suffers from limited imaging penetration. We present an alternative approach, photoacoustic imaging (PAI) of biopotentials, that relies on transient absorption of light by voltage-sensitive probes and subsequent generation/detection of ultrasound. PAI-based voltage imaging approach can offer the same advantages as the fluorescence imaging in terms of sensitivity and molecular specificity, but it also can significantly extend the imaging depth. This study investigated the feasibility of photoacoustically visualizing biopotentials in rat pheochromocytoma (PC12) cells tagged with voltage-sensitive styrylpyridinium dye, RH795. A change in the intramembrane potential was induced in PC12 cells by adding tetraphenylborate (TPB) to the cell culture. A custom-made absorption spectrophotometer was used to verify the change in optical absorption of RH795 dye as a result of TPB-induced electrical fields. Absorption spectra recorded before and after the addition of 100 μM TPB exhibited a wavelength shift of the absorption peak (520 nm to 560 nm) as well as an increase in the overall magnitude of absorption in the wavelength range of 500-1000 nm. Photoacoustic measurements of TPB-induced change in membrane voltage were recorded at an excitation wavelength of 700 nm. Photoacoustic-signal amplitude increased monotonically with time after the addition of TPB, which was consistent with the spectrophotometer measurements.

10051-33, Session 8

Mapping cell-specific functional connections in the mouse brain using ChR2-evoked hemodynamic signals

Adam Q. Bauer, Andrew Kraft, Washington Univ. School of Medicine in St. Louis (United States); Grant A. Baxter, Michael R. Bruchas, Jin-Moo Lee, Washington Univ. School of Medicine (United States); Joseph P. Culver, Washington Univ. School of Medicine in St. Louis (United States)

Functional magnetic resonance imaging (fMRI) has transformed our understanding of the brain's functional organization. However, mapping subunits of a functional network using hemoglobin alone presents several disadvantages. Evoked and spontaneous hemodynamic fluctuations reflect ensemble activity from several populations of neurons making it difficult to discern excitatory vs inhibitory network activity. Still, blood-based methods of brain mapping remain powerful because hemoglobin provides endogenous contrast in all mammalian brains. To add greater specificity to hemoglobin assays, we integrated optical intrinsic signal(OIS) imaging with optogenetic stimulation to create an Opto-OIS mapping tool that combines the cell-specificity of optogenetics with label-free, hemoglobin imaging. Before mapping, titrated photostimuli determined which stimulus parameters elicited linear hemodynamic responses in the cortex. Optimized stimuli were then scanned over the left hemisphere to create a set of optogenetically-defined effective connectivity (Opto-EC) maps. For many sites investigated, Opto-EC maps exhibited higher spatial specificity than those determined using spontaneous hemodynamic fluctuations. For example, resting-state functional connectivity (RS-FC) patterns exhibited widespread ipsilateral connectivity while Opto-EC maps contained distinct short- and long-range constellations of ipsilateral connectivity. Further, RS-FC maps were usually symmetric about midline while Opto-EC maps displayed more heterogeneous contralateral homotopic connectivity. Both Opto-EC and RS-FC patterns were compared to mouse connectivity data from the Allen Institute. Unlike RS-FC maps, Thy1-based maps collected in awake, behaving mice closely recapitulated the connectivity structure derived using ex vivo anatomical tracer methods. Opto-OIS mapping could be a powerful tool for understanding cellular and molecular contributions to network dynamics and processing in the mouse brain.

10051-34, Session 8

Wide area mapping of resting state functional connectivity at microvascular resolution with multi-contrast optical imaging

Janaka Senarathna, Darian Hadjiabadi, Namrata Batra, Stacy Gil, Johns Hopkins Univ. (United States); Nitish V. Thakor, National Univ. of Singapore (United States) and Johns Hopkins Univ. (Singapore); Arvind P. Pathak, Johns Hopkins Univ. (United States)

Different brain regions exhibit complex information processing even at rest. Therefore, assessing temporal correlations between regions permits task-free visualization of their 'resting state connectivity'. Although functional MRI (fMRI) is widely used for mapping resting state connectivity in the human brain, it is not well suited for 'microvascular scale' imaging in rodents because of its limited spatial resolution. Moreover, co-registered cerebral blood flow (CBF) and total hemoglobin (HbT) data are often unavailable in conventional fMRI experiments. Therefore, we built a customized system that combines laser speckle contrast imaging (LSCI), intrinsic optical signal (IOS) imaging and fluorescence imaging (FI) to generate multi-contrast functional connectivity maps at a spatial resolution of 10 ?m. This system comprised of three illumination sources: a 632 nm HeNe laser (for LSCI), a

570 nm ± 5 nm filtered white light source (for IOS), and a 473 nm blue laser (for FI), as well as a sensitive CCD camera operating at 10 frames per second for image acquisition. The acquired data enabled visualization of changes in resting state neurophysiology at microvascular spatial scales. Moreover, concurrent mapping of CBF and HbT-based temporal correlations enabled in vivo mapping of how resting brain regions were linked in terms of their hemodynamics. Additionally, we complemented this approach by exploiting the transit times of a fluorescent tracer (Dextran-FITC) to distinguish arterial from venous perfusion. Overall, we demonstrated the feasibility of wide area mapping of resting state connectivity at microvascular resolution and created a new toolbox for interrogating neurovascular function.

10051-35, Session 8

Development of a multi exposure speckle imaging for mice brain imaging

Haleh Soleimanzad, Univ. Paris-Sud (France); Hirac Gurden, Univ. Paris Denis Diderot, CNRS UMR8251 (France); Frederic Pain, Univ. Paris-Sud (France)

A multi exposure speckle imaging system has been developed and characterized for wide field blood flow imaging of the mice cortex. We report on the choice and validation of the system components including coherent light source, fast CMOS camera and acousto-optic modulator. We have characterized its performances using microfluidic phantoms composed of channels with diameters ranging from 10 to 200 microns where blood mimicking fluids speed is set between 1mm/s and 1cm/s. The first in vivo imaging attempts in the mice brain will be presented

10051-36, Session 9

Depth monitoring of cerebral hemodynamic changes using Monte Carlo-based probabilistic photon paths

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Accurate and efficient reconstruction of hemodynamic changes is an important step towards the implementation of NIRS as an enhanced clinical tool for understanding oxygenation changes at various depths within the brain. Depth information could provide insight on how oxygen transported to the tissue. For this work, we ran Monte Carlo simulations to develop sensitivity profiles for various source-detector separations. The source-detector separations were based on our custom built 108 channel NIRS probe and consisted of separations of 15 mm, 30 mm, 36 mm, and 45 mm. We used the mesh-based Monte Carlo program MMCLAB (Fang et al. 2010) to acquire the sensitivity profiles. The sensitivity profiles consisted of a tetrahedral mesh which was converted to a regular grid in three-dimensional space. Then, the structural tensor was calculated for each voxel and the Hamilton-Jacobi equation was solved anisotropically for the tensor volume. As the result, the distance map was in same space as the calculated tensor volume. Using this distance map, we modeled the probabilistic path of photons. We then weighted the hemodynamic changes acquired by our NIRS probe according to the probabilistic path to reconstruct hemodynamic changes in the prefrontal area of the brain.

10051-37, Session 9

A global metric to detect motion artifacts in optical neuroimaging data

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As with other imaging modalities, motion induces artifacts that can significantly corrupt optical neuroimaging data. While multiple methods have been developed for motion detection in individual NIRS measurement channels, the large measurement numbers present in multichannel fNIRS or high-density diffuse optical tomography (HD-DOT) systems, create an opportunity for detection methods that integrate over the entire field of view. Here, we leverage the inherent covariance among multiple NIRS measurements after pre-processing, to quantify motion artifacts by calculating the global variance in the temporal derivative (GV-TD) across all measurements (e.g. from the temporal derivative of each time-course, the method calculates root mean square across all measurements for each time point). This calculation is fast, automated, and identifies motion by incorporating global aspects of data instead of individual channels. To test the performance, we designed an experimental paradigm that intermixed controlled epochs of motion artifact with relatively motion-free epochs during a block design hearing-words language paradigm using a previously described HD-DOT system. We categorized 348 blocks by sorting the blocks based on the maximum of their GV-TD time-courses. Our results show that with a modest thresholding of the data, wherein we keep data with 0.66 of the full data set average GV-TD, we obtain a ~50% increase in the signal-to-noise. With noisier data, we expect the performance gains to increase. Further, the impact on resting state functional connectivity may also be more significant. In summary, a censoring threshold based on the GV-TD metric provides a fast and direct way for identifying motion artifacts.

10051-38, Session 9

Improved accuracy of brain oxygen metabolism measurements using multi-distance diffuse correlation spectroscopy and near infrared spectroscopy together with a Monte Carlo light transport model

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Diffuse correlation spectroscopy (DCS) is being employed alongside near-infrared spectroscopy (NIRS) measurements to track the cerebral oxygen metabolic rate (CMRO2). However, both techniques employ diffusely reflected light that has traveled mostly through extracerebral tissues. Recent studies indicate that depth sensitivity profiles are different for NIRS vs DCS measurements, with DCS appearing to be more sensitive to the brain than NIRS methods for a given source-detector separation. This mismatch can lead to erroneous conclusions with respect to the amount and perhaps even the direction of change in CMRO2. Recently, our group and others have demonstrated the use of Monte Carlo (MC) based multi-layer, multi-distance fitting, which offers increased accuracy for complex tissue structures such as the adult brain.

In this paper we employ a Monte Carlo light transport model based on a realistic head geometry that can be derived from MRI scans (if available) or approximated from head shape measurements. We consider DCS and CW-NIRS measurements taken at two or more distances and analyze simulated data generated using a fully segmented adult brain MRI scan. Through simulations, we explore the improvements offered by our method vs. processing the same measurements with a semi-infinite diffusion model and estimate the impact of errors in geometry and optical properties on relative blood flow and CMRO2 changes.

10051-39, Session 9

Correlation between VEP and hemodynamics in visual cortex

Xin Zhang, Institute of Automation (China)

Brain functional activity involves complex cellular, metabolic, and vascular chain reactions, making it difficult to clarify its mechanism. However, clinical demanding of understanding the mechanism is dramatically increasing as brain diseases may due to an abnormal correlation of certain cerebral cortex. As electroencephalography (EEG) and functional near infrared spectroscopy (fNIRS) could be easily used in clinics. They have also been combined into a multimodal neuroimaging method that captures both electrophysiological and hemodynamic information to explore the spatiotemporal characteristics of brain activity. Because of the significance of visually evoked functional activity in clinical applications, we are focusing on the visual evoked potential (VEP) and are supposed to clarify its relationship with the hemodynamic response. Relatively few studies have investigated the influence of latency, which has been frequently used to diagnose visual diseases, on the hemodynamic response. Moreover, because the latency and the amplitude of VEPs have different roles in coding visual information, investigating the relationship between latency and the hemodynamic response should be helpful. In this study, checkerboard reversal tasks with graded contrasts were used to evoke visual functional activity. Both EEG and fNIRS were employed to investigate the relationship between neuronal electrophysiological activities and the hemodynamic responses. The VEP amplitudes were linearly correlated with the hemodynamic response, but the VEP latency showed a negative linear correlation with the hemodynamic response.

Conference 10052: Optogenetics and Optical Manipulation

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10052-1, Session 1

Super-duper chemiluminescent proteins applicable to wide range of bioimaging *(Keynote Presentation)*

Takeharu Nagai, Osaka Univ. (Japan)

No Abstract Available

10052-2, Session 1

Spatial control of in vivo optogenetic light stimulation and recording via an imaging fiber bundle

Javier I. Suárez, Parijat Sengupta, Jonathan Guo-Han Mun, Justin Rhodes, Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Light delivery in in vivo optogenetic applications are typically accomplished via a single multimode fiber that diffuses light over a large area of the brain, and relies heavily on the spatial distribution of transfected light-sensitive neurons for targeted control.

In our investigations, an imaging fiber bundle (Schott, 1534702) containing 4,500 individual fibers, each with a diameter of 7.5 μm, and an overall outer bundle diameter of 530 μm, was used as the conduit for light delivery and optical recording/imaging in neuron cultures and in in vivo mouse brain. We demonstrated that the use of this fiber bundle, in contrast to a single multimode fiber, allowed for individually-addressable fibers, spatial selectivity at the stimulus site, precise control of light delivery, and full field-of-view imaging and/or optical recordings of neurons. An objective coupled the two continuous wave diode laser sources (561 nm/488 nm) for stimulation and imaging into the proximal end of the fiber bundle while a set of galvanometer-scanning mirrors was used to couple the light stimulus to distinct fibers. A micro lens aided in focusing the light at the neurons. In vivo studies utilized C1V1(E122T/E162T)-TS-p2A-mCherry (Karl Deisseroth, Stanford) and GCaMP6s transgenic mice (Jackson Labs) for this all-optical approach.

Our results demonstrate that imaging fiber bundles provide superior control of spatial selectivity of light delivery to specific neurons, and function as a conduit for optical imaging and recording at the in vivo site of stimulation, in contrast to the use of single multimode fibers that diffusely illuminate tissue and lack in vivo imaging capabilities.

10052-3, Session 1

Using stereotactic brain atlases for small rodents and nonhuman primates for optrode array customization.

Ronnie Boutte, Northrop Grumman Mission Systems (United States) and Univ. of Utah (United States); Sam Merlin, The Univ. of Utah (United States) and John A. Moran Eye Ctr. (United States); Trent Parry, Steve Blair, The Univ. of Utah (United States)

As the optogenetic field expands its need to target, with high specificity, only grows more crucial. This work will show a method for customizing soda-lime glass optrode arrays so that fine structures within the brains of small rodents and nonhuman primates can be optically interrogated deep below the outer cortex. Stereotactic atlases are readily available for mice, rats, marmosets, macaques, and several other animals that are suitable models for understanding the human brain. The stereotaxic coordinate system (SCS) serves as good foundation for the identification of optogenetic tissue targeting by identifying internal brain structures in relation to cranial anatomy: Bregma, Interaural Line, and Midline as measured from the Sagittal Suture. The SCS is used to design an 8X6 optrode array for interrogating neocortical layers of the primate (Macaca Fascicularis).

An 8X6 array is customized for optrode length (800um), optrode width (75um), optrode pitch (400um), backplane thickness (500um), and overall form factor 3.45mm x 2.65mm. The 800um long optrode is capable of stopping at the boundary of layer III and layer IV. Orthogonally diced optrodes ensures straight wave guides reducing the amount of TIR loss, while over fire-polishing eliminates all sharpened edges to reduce the amount of tissue damage. By varying the optical source power, these arrays are capable of illuminating the laminar layers I thru VI by delivering light to layers I and II through the backplane and deeper into tissue through the optrode.

10052-4, Session 2

Optical detection of single action potential spikes in mammalian neuronal network

Subrata Batabyal, Sarmishtha Satpathy, Loan Bui, Young-Tae Kim, The Univ. of Texas at Arlington (United States); Samarendra K. Mohanty, Nanoscope Technologies, LLC (United States); Digant P. Davé, The Univ. of Texas at Arlington (United States)

Electrophysiology techniques are the gold standard in neuroscience for studying functionality of a single neuron to a complex neuronal network. However, electrophysiology techniques are not flawless, they are invasive nature, procedures are cumbersome to implement with limited capability of being used as a high-throughput recording system. Also, long term studies of neuronal functionality with aid of electrophysiology is not feasible. Non-invasive stimulation and detection of neuronal electrical activity has been a long standing goal in neuroscience. Introduction of optogenetics has ushered in the era of non-invasive optical stimulation of neurons, which is revolutionizing neuroscience research. Optical detection of neuronal activity that is comparable to electro-physiology is still elusive. A number of optical techniques have been reported recording of neuronal electrical activity but none is capable of reliably measuring action potential spikes that is comparable to electro-physiology. Optical detection of action potential with voltage sensitive fluorescent reporters are potential alternatives to electrophysiology techniques. The heavily rely on secondary reporters, which are often toxic in nature with background fluorescence, with slow response and low SNR making them far from ideal. The detection of one shot (without averaging)-single action potential in a true label-free way has been elusive so far. In this report, we demonstrate the optical detection of single neuronal spike in a cultured mammalian neuronal network without using any exogenous labels. To the best of our knowledge, this is the first demonstration of label free optical detection of single action potentials in a mammalian neuronal network, which was achieved using a high-speed phase sensitive interferometer. We have carried out stimulation and inhibition of neuronal firing using Glutamate and Tetrodotoxin respectively to demonstrate the different outcome (stimulation and inhibition) revealed in optical signal. We hypothesize that the interrogating optical beam is modulated during neuronal firing by electro-motility driven membrane fluctuation in conjunction with electrical wave propagation in cellular system.

10052-5, Session 2

Deep brain single optical fiber fluorescence imaging

Israel Delarosa, Mehdi Azimipour, Ramin Pashaie, Univ. of Wisconsin-Milwaukee (United States)

This project strives to address the need for the development of a practical and cost-efficient device to measure the density and spatial distribution of fluorescence molecules in deep brain regions. This goal is achieved by proposing a novel optoelectronic design that uses only a relatively thin penetrating and rotating side-firing fiber to scan the brain tissue and collect data sets. These data sets are processed in a computer to reconstruct images displaying the distribution of fluorescence molecules in a cylindrical volume surrounding the fiber and algorithms are applied for modeling light-tissue interaction in the brain. We expect to achieve a radial penetration depth of ~1.0mm around the optical axis of the fiber with axial resolution of ~100um or better, while each complete scan takes time in the range of several minutes. The main benefits of this design are the simplicity of the hardware and experimental protocols since the procedure is only minimally invasive and image development is performed by computer algorithms and image reconstruction subroutines.

10052-6, Session 2

Micro-device combining electrophysiology and optical imaging for functional brian monitoring in freely moving animals

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In this study, we developed a new standalone micro-device combining EEG and LSCI for monitoring the cerebral blood flow and neural activities with more feasibility for freely moving animals.The micro-device is fully standalone without fiber and cable connections. It can be further combined with the optogenetic tools by adequately adding specific stimulation light sources for close-loop control and monitoring of brain circuits.

10052-7, Session 2

Integration of flexible inorganic light emitting diodes and transparent PEDOT:PSS/Parylene C for simultaneous optogenetics and electrocorticography

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Electrocorticography (ECoG) is a powerful tool for direct mapping of local field potentials from the brain surface. Progress in development of high-fidelity materials such as poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) on thin conformal substrates such as parylene C enabled intimate contact with cortical surfaces and higher quality recordings from small volumes of neurons. Meanwhile, stimulation of neuronal activity is conventionally accomplished with electrical microstimulation and transcranial magnetic stimulation that can be combined with ECoG to form the basis of bidirectional neural interface. However, these stimulation mechanisms are less controlled and primitively understood on the local and cellular levels. With the advent of optogenetics, the localization and specificity of neuronal stimulation and inhibition is possible. Therefore, the development of integrated devices that can merge the sensitivity of ECoG or depth recording with optogenetic tools can lead to newer frontiers in understanding the neuronal activity.

Herein, we introduce a hybrid device comprising flexible inorganic LED arrays integrated PEDOT:PSS/parylene C microelectrode arrays for high resolution bidirectional neuronal interfaces. The flexible inorganic LEDs have been developed by the metal-organic vapor phase epitaxy of position-controlled GaN microLEDs on ZnO nanostructured templates pre-grown at precise locations on a graphene layer. By transferring it onto the microelectrode arrays, it can provides the individual electrical addressability by light stimulation patterns. We will present experimental and simulation results on the optoelectronic characteristics and light activation capability of flexible microLEDs and their evaluation in vivo.

10052-8, Session 3

Organic LEDs as biocompatible light sources for optogenetics *(Invited Paper)*

Anja Steude, Andrew Morton, Caroline Murawski, Emily C. Witts, Gareth B. Miles, Stefan R. Pulver, Malte C. Gather, Univ. of St. Andrews (United Kingdom)

Future advances in optogenetic stimulation and silencing will require biocompatible light sources with spatially and temporally controlled illumination. Here, we show that organic light-emitting diodes (OLEDs) are highly attractive in this context, in particular due to their low toxicity, fast switching, high brightness, and ability to provide patterned illumination with very high spatial resolution.

In initial proof-of-concept demonstrations, we used OLED microdisplays to control the light-activated locomotion (phototaxis) of the green alga Chlamydomonas reinhardtii [1]. The microdisplays consisted of a silicon chip containing electronics that addresses > 105 individual top-emitting OLED pixels with μm dimensions deposited directly on the chip.

More recently, we showed OLED-mediated optogenetic control on both the single cell level [2] and using Drosophila larvae [3]. In comparison to naturally occurring light-sensitive systems, optogenetics is often relatively light inefficient, requiring optical intensities on the order of mW/mm2 for robust activation. Our work shows that, maybe somewhat against common expectation, OLEDs reliably achieve such intensities at acceptable voltages when using state-of-the-art fluorescent pin devices.

[1] A Steude et al, “Controlling the movement of single live cells with high density arrays of microscopic OLEDs” Advanced Materials 27, 7657-7661 (2015)

[2] A Steude et al, “Arrays of Microscopic Organic LEDs for High Resolution Optogenetics ChR2 activation with OLED microarrays” Science Advances 2, e1600061 (2016)

[3] A Morton et al, “High-brightness organic light-emitting diodes for optogenetic control of Drosophila locomotor behaviour” (in press)

10052-9, Session 3

Cardiac optogenetic pacing in Drosohpila melanogaster using red-shifted opsins

Jing Men, Lehigh Univ. (United States); Airong Li, Massachusetts General Hospital (United States); Jason Jerwick, Lehigh Univ. (United States); Rudolph E. Tanzi, Massachusetts General Hospital (United States); Chao Zhou, Lehigh Univ. (United States)

Electrical pacing is the current gold standard for investigation of mammalian

cardiac electrical conduction systems as well as for treatment of certain cardiac pathologies. However, this method requires an invasive surgical procedure to implant the pacing electrodes. Recently, optogenetic pacing has been developed as an alternative, non-invasive method for heartbeat pacing in animals. It induces heartbeats by shining pulsed light on transgene-generated microbial opsins which in turn activate light gated ion channels in animal hearts. However, commonly used opsins, such as channelrhodopsin-2 (ChR2), require short light wavelength stimulation (475 nm), which is strongly absorbed and scattered by tissue. Here, we expressed recently engineered red-shifted opsins, ReaChR and CsChrimson, in the heart of a well-developed animal model, Drosophila melanogaster, for the first time. Optogenetic pacing was successfully conducted in both ReaChR and CsChrimson flies at their larval, pupal, and adult stages using 617 nm excitation light pulse, enabling a much deeper tissue penetration compared to blue stimulation light. A customized high speed and ultrahigh resolution OCM system was used to non-invasively monitor the heartbeat pacing in Drosophila. Compared to previous studies on optogenetic pacing of Drosophila, higher penetration depth of optogenetic excitation light was achieved in opaque late pupal flies. Lower stimulating power density is needed for excitation at each developmental stage of both groups, which improves the safety of this technique for heart rhythm studies.

10052-10, Session 3

Reducing peak temperatures during infrared inhibition of neural potentials

Jeremy B. Ford, Mohit Ganguly, Vanderbilt Univ. (United States); Michael W. Jenkins, Hillel J. Chiel, Case Western Reserve Univ. (United States); E. Duco Jansen, Vanderbilt Univ. (United States)

Pulsed infrared (IR) light has been used in multiple animal and computational models to inhibit neural activity. Duke et al. reported a complete inhibition associated temperature increase of ~8 degrees Celsius in Aplysia californica buccal nerve 2 (BN2). Inhibition paradigms thus far have used a single optical fiber to deliver IR, resulting in a single hotspot within the nerve. Past acute studies show no change in physiology. For some clinical applications requiring prolonged inhibition, lower temperatures may increase the safety margin. One proposed method for decreasing peak temperatures is to use a lower power over a greater area, effectively heating the nerve more evenly. Preliminary computational modeling suggests that using two axially adjacent optical fibers reduces peak temperature rise by about 40%. This trend is being validated in vitro in A. californica. Buccal ganglia were dissected out, and suction electrodes were applied to record BN2 spontaneous activity. Two optical fibers (core diameters= 400 μm) were epoxied directly together (d= 440 μm between fiber centers), and polished together. These were used to simultaneously apply IR light from two diode lasers (?=1875nm) to the nerve. Power and temperature values required for inhibition using a single fiber were compared to those from the two axially adjacent fibers. Peak temperatures were thermally imaged post-hoc in a tissue phantom. By reducing peak temperatures, neural block using IR light will subject nerves to lower maximum temperatures and thus enhance IR inhibition's clinical utility.

10052-11, Session 4

Optimizing thermal inhibition of nerves using an integrated computational model

Mohit Ganguly, Vanderbilt Univ. (United States); Michael W. Jenkins, Hillel J. Chiel, Case Western Reserve Univ. (United States); E. Duco Jansen, Vanderbilt Univ. (United States)

Infrared (IR) lasers (with an optical penetration depth of several hundred microns, e.g.. 1.87 μm) are capable of inducing a thermally mediated nerve block in Aplysia and rat nerves. This block is spatially precise, reversible and does not alter neural physiology when removed. We present a computational model for studying the interaction of infrared light with an unmyelinated axon. The ability to combine the photothermal response of nerve tissue with the electrical response of the nerve axon in silico will enhance explorations of parameter space and guide future feasibility studies of selectively blocking pain conduction fibers in humans. This model can compute the electrical response of unmyelinated axons due to IR laser-induced temperature increases on the nerve. We combine an optical-thermal distribution model, which consists of a Monte Carlo light distribution and a Pennes' bio-heat transfer model, with a modified temperature-dependent Hodgkin-Huxley ion-channel model. We want to identify a set of laser parameters (number of laser sources, wavelengths, distances of laser source from tissue, fluence, pulse duration, repetition rate, and beam area) that will enable us to achieve conduction block in the nerve with a minimal rise in temperature. As an example, the model has predicted that the length of illumination along the nerve is an important parameter for block. We will also explore these parameters to increase efficiency and safety (i.e., reduce thermal load) to the tissue.

10052-12, Session 4

Reconfigurable visible nanophotonic switch for optogenetic applications

Aseema Mohanty, Columbia Univ. (United States) and Cornell Univ. (United States); Qian Li, Cold Spring Harbor Lab. (United States); Mohammad Amin Tadayon, Gaurang R. Bhatt, Columbia Univ. (United States); Jaime Cardenas, Univ. of Rochester (United States) and Columbia Univ. (United States); Steven A. Miller, Columbia Univ. (United States) and Cornell Univ. (United States); Adam Kepecs, Cold Spring Harbor Lab. (United States); Michal Lipson, Columbia Univ. (United States)

High spatiotemporal resolution deep-brain optical excitation for optogenetics would enable activation of specific neural populations and in-depth study of neural circuits. Conventionally, a single fiber is used to flood light into a large area of the brain with limited resolution. The scalability of silicon photonics could enable neural excitation over large areas with single-cell resolution similar to electrical probes. However, active control of these optical circuits has yet to be demonstrated for optogenetics.

Here we demonstrate the first active integrated optical switch for neural excitation at 473 nm, enabling control of multiple beams for deep-brain neural stimulation. Using a silicon nitride waveguide platform, we develop a cascaded Mach-Zehnder interferometer (MZI) network located outside the brain to direct light to 8 different grating emitters located at the tip of the neural probe. We use integrated platinum microheaters to induce a local thermo-optic phase shift in the MZI to control the switch output. We measure an ON/OFF extinction ratio of >8dB for a single switch and a switching speed of 20 microseconds. We characterize the optical output of the switch by imaging its excitation of fluorescent dye.

Finally, we demonstrate in vivo single-neuron optical activation from different grating emitters using a fully packaged device inserted into a mouse brain. Directly activated neurons showed robust spike firing activities with low first-spike latency and small jitter. Active switching on a nanophotonic platform is necessary for eventually controlling highly-multiplexed reconfigurable optical circuits, enabling high-resolution optical stimulation in deep-brain regions.

10052-13, Session 4

Optical cell stimulation for neuronal excitation

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Development (Germany); Patrick Heeger, Leibniz Univ. Hannover (Germany) and NIFE - Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (Germany); Mitsuhiro Terakawa, Keio Univ. (Japan); Alexander Heisterkamp, Leibniz Univ. Hannover (Germany) and NIFE - Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (Germany); Tammo Ripken, Laser Zentrum Hannover e.V. (Germany); Dag Heinemann, Laser Zentrum Hannover e.V. (Germany) and NIFE - Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (Germany)

Optical manipulation of cellular functions represents a growing field in biomedical sciences. The possibility to modulate specific targets with high spatial and temporal precision in a contactless manner allows a broad range of applications. Here, we present a study on stimulation of neuronal cells by optical means. As a long-term objective, we seek to improve the performance of current electric neurostimulation, especially in the context of cochlear implants. Firstly, we tested a gold nanoparticle mediated approach to modulate transmembrane conductivity by irradiation using a picosecond pulsed Nd:YAG laser at 532 nm for 40 ms in a neuroblastoma cell line (N2A) and primary murine neurons. The light absorption leads to a rapid temperature increase of the gold nanoparticles, which can induce an increased permeabilisation of the cellular membrane. Calcium transients were recorded as an indicator of neuronal activity. Although calcium signals were reliably detected upon laser irradiation, the temporal behavior did not resemble action potentials. The origin of these signals was investigated by an inhibitor study. These results indicate calcium induced calcium release (CICR) as the major source of the calcium transients. Consecutively, we tested alternative approaches for cell stimulation, such as glutamate release and optogenetics, and evaluated the potential of these methods for the application in a cochlear implant. Compared to the gold nanoparticle approach, both techniques induce less cellular stress and reliably produce action potentials.

10052-14, Session 4

Measurement of light transmission and irradiance in brain tissue in vivo

John J. Macklin, Austin R. Graves, Adam W. Hantman, Katie C. Bittner, Howard Hughes Medical Institute (United States)

Optogenetic experiments require light delivery, typically using fiber optics, to light-gated ion channels genetically targeted to specific brain regions. Understanding where light is—and isn't—in an illuminated brain can be a confounding factor in designing experiments and interpreting results. While the transmission of light, i.e. survival of forward-directed and forward–scattered light, has been extensively measured in vitro, light scattering can be significantly different in vivo due to blood flow and other factors. To measure irradiance in vivo, we constructed a pipette photodetector tipped with fluorescent quantum dots that function as a light transducer. The quantum dot fluorescence is collected by a waveguide and sent to a fiber-coupled spectrometer. The device has a small photo-responsive area (~ 10 um x 15 um), enabling collection of micron-resolution irradiance profiles, and can be calibrated to determine irradiance with detection limits of 0.001 mW/mm2. The photodetector has the footprint of a micro-injection pipette, so can be inserted into almost any brain region with minimal invasiveness. With this detector, we determined transverse and axial irradiance profiles in mice across multiple brain regions at 5 source wavelengths spanning the visible spectrum. This profile data is compared to in vitro measurements obtained on tissue slices, and provides a means to derive scattering coefficients for specific brain regions in vivo. The detector is straightforward to fabricate and calibrate, is stable in air storage > 9 months, and can be easily installed in an electrophysiology setup, thereby enabling direct measurement of light spread under conditions used in optogenetics experiments.

10052-15, Session 4

3D Monte Carlo model with direct photon flux recording for optimal optogenetic light delivery

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Configuring light power from the optical fiber is an essential first step in planning in-vivo optogenetic experiments. However, a detailed understanding of the light emitted from the fiber in the heterogeneous 3D brain remains limited due to the absence of an appropriate modeling tool for this purpose. We present a mesh-based 3D Monte Carlo model that record the direct photon flux for applications in fiber-optic-optogenetic neural stimulation. Our method is aimed to maximize near-field recording efficiency by considering the close proximity between a source of stimulation and a neural target in optogenetic applications. Unlike the conventional weight-loss approach, the photon trajectory is directly recorded in the separate volumetric grid planes and the photons under absorption is immediately terminated without reduction of photon weight for the near-source efficiency gain. We investigated the light emitted from optical fibers in 3D using various methods, and we demonstrated that the diffusion theory precludes accurate estimates of light intensity for typical optogenetic applications due to the close proximity between an optical fiber and neural target. Our method can be applied to design optimal light delivery conditions for precise optogenetic manipulation by considering the fiber output power and optimized fiber-to-target distance, and tissue heterogeneity. Source code for both of heterogeneous and homogeneous brain is publically available for download.

10052-16, Session 5

Photovoltaic restoration of sight in rodents with retinal degeneration *(Keynote Presentation)*

Daniel V. Palanker, Stanford Univ. (United States)

To restore vision in patients who lost their photoreceptors due to retinal degeneration, we developed a photovoltaic subretinal prosthesis which converts light into pulsed electric current, stimulating the nearby inner retinal neurons. Visual information is projected onto the retina by video goggles using pulsed near-infrared (~900nm) light. This design avoids the use of bulky electronics and wiring, thereby greatly reducing the surgical complexity. Optical activation of the photovoltaic pixels allows scaling the implants to thousands of electrodes, and multiple modules can be tiled under the retina to expand the visual field.

We found that similarly to normal vision, retinal response to prosthetic stimulation exhibits flicker fusion at high frequencies (>20Hz), adaptation to static images, and non-linear summation of subunits in the receptive fields. Photovoltaic arrays with 70um pixels restored visual acuity up to a single pixel pitch, which is only two times lower than natural acuity in rats. If these results translate to human retina, such implants could restore visual acuity up to 20/250. With eye scanning and perceptual learning, human patients might even cross the 20/200 threshold of legal blindness. In collaboration with Pixium Vision, we are preparing this system (PRIMA) for a clinical trial. To further improve visual acuity, we are developing smaller pixels – down to 40um, and on 3-D interface to improve proximity to the target neurons. Scalability, ease of implantation and tiling of these wireless modules to cover a large visual field, combined with high resolution opens the door to highly functional restoration of sight.

10052-17, Session 5

Short infrared laser pulses block action potentials in neurons

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Short infrared laser pulses have many physiological effects on cells including the ability to stimulate action potentials in neurons. Here, we show that short infrared laser pulses can also reversibly block action potentials. Primary rat hippocampal neurons were transfected with the Optopatch2 plasmid, which contains both a blue-light activated channel rhodopsin (CheRiff) and a red-light fluorescent membrane voltage reporter, QuasAr2. This optogenetic platform allows robust stimulation and recording of action potential activity in neurons in a non-contact, low noise manner. For all experiments, QuasAr2 was imaged continuously on a wide-field fluorescent microscope using a Krypton laser (647 nm) as the excitation source and an EMCCD camera operating at 1000 Hz to collect emitted fluorescence. A co-aligned Argon laser (488 nm, 5 ms at 10 Hz) provided activation light for CheRiff. A 200 μm fiber delivered infrared light locally to the target neuron. Reversible action potential block in neurons was observed following a short infrared laser pulse (0.26-0.96 J/cm2; 1.37-5.01 ms; 1869 nm), with the block persisting for more than 1 s with exposures greater than 0.69 J/cm2. Action potential block was sustained for 30 s with repeated short infrared laser pulses at 1-7 Hz. Full recovery of neuronal activity was observed 5-30s post-infrared exposure. These results indicate that optogenetics provides a robust platform for the study of action potential block and that short infrared laser pulses can be used for non-contact, reversible action potential block.

10052-18, Session 5

Active implant for optoacoustic natural sound enhancement

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This paper summarizes the results of an EU project called ACTION: ACTive Implant for Optoacoustic Natural sound enhancement. The project is based on a recent discovery that relatively low levels of pulsed infrared laser light are capable of triggering activity in hair cells of the partially hearing (hearing impaired) cochlea and vestibule. The aim here is the development of a self-contained, smart, highly miniaturized system to provide optoacoustic stimuli directly from an array of miniature light sources in the cochlea. Optoacoustic compound action potentials (oaCAPs) are generated by the light source fully inserted into the unmodified cochlea. Previously, the same could only be achieved with external light sources connected to a fiber optic light guide or with optogenetically modified cells. This feat is achieved by integrating custom made VCSEL arrays at a wavelength of about 1550 nm onto small flexible substrates. The laser light is collimated by a specially designed silicon-based ultra-thin lens (165 um thick) to get the energy density required for the generation of oaCAP signals. A dramatic miniaturization of the packaging technology is also required. A long term biocompatible and hermetic sapphire housing with a size of less than a 1 cubic millimeter and miniature Pt/PtIr feedthroughs is developed, using a low temperature laser assisted process for sealing. A biofouling thin film protection layer is developed to avoid protein adsorption and cell growth on the system. Long term hermeticity is proven by He and dedicated FTIR leak testing methods.

10052-19, Session 6

Neural responses of rat cortical layers due to infrared neural stimulation

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Infrared neural stimulation (INS) is a label-free method for eliciting neural activity with high spatial selectivity in mammalian models. While there has been an emphasis on INS research towards applications in the peripheral nervous system and the central nervous system (CNS), the biophysical mechanisms by which INS occurs remains unresolved. In the rat CNS, INS has been shown to elicit and inhibit neural activity, evoke calcium signals that are dependent on glutamate transients and astrocytes, and modulate inhibitory GABA currents. So far, in vivo experiments have been restricted to layers I and II of the rat cortex due to light absorption, which consists mainly of astrocytes, inhibitory neurons, and dendrites from deeper excitatory neurons. Deeper cortical layers (III-VI) have vastly different cell type composition, consisting predominantly of excitatory neurons which can be targeted for therapies such as deep brain stimulation. The neural responses of deeper cortical cells have not been well defined. Acute thalamocortical brain slices and cell cultures will allow us to analyze the effects of INS on various components of the cortex, including different cortical layers and cell populations. In this study, we present the neural responses of different cortical cell types due to exposure to pulsed infrared light (?=1.875 μm, 200 μm fiber, 200 Hz) using electrophysiological and optical techniques. These responses will lead to a finer characterization of the parameter space for different cortical cell types and may contribute to understanding the cell populations that are important for allowing optical stimulation of neurons in the CNS.

10052-20, Session 6

Spectral-temporal modulation of supercontinuum generation for optogenetics

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By combining optical and genetic methods, optogenetics has become a very important tool in neuroscience research for manipulating neuron activities. The rapid development of novel opsins and fluorescent indicators has introduced a large palette of biochemical probes for optogenetic stimulation and cellular imaging, which makes the all-optical neural circuit excitation and neural activity recording possible. Compared to visible-light illumination, two-photon excitation and imaging avoids the crosstalk from optogenetic probes and calcium sensors, and provides for deeper penetration and higher spatial-temporal resolution for single-cell-level precise manipulation. Two-photon interactions frequently necessitate the use of high-power sources with narrow bandwidth outputs. Although tunable sources, such as the titanium-sapphire laser, offer some degree of flexibility, multiple bulky and expensive lasers are required for simultaneous two-photon optogenetic stimulation and calcium imaging. Here, we propose to use fiber-based supercontinuum generation as a broadband coherent light source for two-photon excitation and imaging. A custom-made

photonic crystal fiber is pumped by a Yb:KYW laser (1041 nm, 220 fs, 80 MHz) to generate a femtosecond output with a wide range of wavelengths, 900 - 1170 nm, which covers most of the two-photon excitation wavelengths of the molecules used in optogenetics, e.g. C1V1-2A-mCherry and GCaMP6s in our study. A pulse shaper is utilized to modulate the phases of partial wavelengths to tailor the temporal shape of the femtosecond pulse, which manipulates the absorption of optogenetic probes and provides a unique approach for controllable optogenetic excitation. Video-rate calcium imaging results suggest that spectral-temporal programmable supercontinuum pulses provide a powerful tool for neural network activity research.

10052-21, Session 6

Analysis of components of compound action potentials in response to infrared laser light

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Many techniques may modulate peripheral nerve activity. Infrared light (IR) can excite or inhibit nerves. Compound action potentials (CAPs) are often measured as an endpoint, focusing on complete block, or overall amplitude reduction. To our knowledge, no standard techniques determine whether CAP sub-components have been modulated. Treatments may alter timing of CAP components as well as blocking them. How can these be distinguished?

We developed a numerical simulation in which extracellularly recorded action potentials were summed, assuming a Gaussian distribution for their onset time. Onset time for sub-populations was delayed (shifting), or amplitudes were reduced to zero (blocking). We demonstrated that area under the rectified curve, divided by the entire duration of the CAP, provided a more stable measure of change than other options (e.g., power). Regions must be selected such that the CAP's individual components do not shift out of the analysis window. The largest reductions in area under the curve due to shifts were ~55% due to destructive interference, which is likely to be much larger than typically observed experimentally. In contrast, blocking components could reduce the area under the curve to zero.

The analysis was applied to sequential nerve stimulations. At every point, variance of the normalized area was computed. Choosing regions of lowest variance across stimulations defined an objective criterion for boundaries between CAP subcomponents. Analysis was applied to IR effects on CAPs recorded in the pleural-abdominal connective of Aplysia californica and musk shrew vagus. Slower conducting CAP subcomponents were selectively blocked before faster subcomponents.

10052-28, Session 7

3D imaging and optogenetics of cortical circuits *(Keynote Presentation)*

Rafael Yuste M.D., Columbia Univ. (United States)

No Abstract Available

10052-23, Session 8

Contralesional homotopic activity negatively influences functional recovery after stroke

Adam Q. Bauer, Andrew Kraft, Grant A. Baxter, Michael R. Bruchas, Jin-Moo Lee, Joseph P. Culver, Washington Univ. School of Medicine in St. Louis (United States)

Recent fcMRI studies examining spontaneous brain activity after stoke have revealed disrupted global patterns of functional connectivity (FC). Interestingly, acute interhemispheric homotopic FC has been shown to be predictive of recovery potential. While substantial indirect evidence also suggests that homotopic brain activity may directly impact recovery, results in humans are extremely varied. A better understanding of how activity within networks functionally-connected to lesioned tissue influences brain plasticity might improve therapeutic strategies. We combine cell-type specific optogenetic targeting with optical intrinsic signal (OIS) imaging to assess the effects of homotopic contralesional activity (specifically in excitatory CamKIIa pyramidal neurons) on FC, cortical remapping, and behavior after stroke. Thirty-one mice were housed in enriched cages for the experiment. OIS imaging was performed before, 1, and 4 weeks after photothrombosis of left forepaw somatosensory cortex (S1fp). On day 1 after stroke, 17 mice were subjected to chronic, intermittent optical stimulation of right S1fp for 10 min, 5 days/week for 4 weeks. New cortical representations of left S1fp appeared in non-stimulated mice at week 1, but not in stimulated mice ($p=0.005$). Evoked responses were comparable in both groups at week 4 ($p=0.57$). Homotopic FC between left and right S1fp regions was equally reduced in both groups ($p=0.012$) at week 1. However, in non-stimulated mice, behavioral performance and FC between right S1fp and left perilesional S1 cortex was significantly higher by 4 weeks compared to stimulated mice ($p=0.009$). Our results suggest that increased homotopic, contralesional activity in excitatory neurons negatively influences spontaneous recovery following ischemic stroke.

10052-24, Session 8

Multi-Characteristics Opsin enabled vision restoration

Weldon Wright, Sanjay Pradhan, Sulgana Bhattacharya, Vasu Mahapatra, Ashutosh Tripathy, Sivakumar Gajjeraman, Samarendra K. Mohanty, Nanoscope Technologies, LLC (United States)

Photodegenerative retinal diseases such as retinitis pigmentosa and dry age related macular degeneration lead to loss of vision in millions of individuals. Currently, no surgical or medical treatment is available though optogenetic therapies are in clinical development. Here, we demonstrate vision restoration using Multi-Characteristics Opsin (MCO) in animal models with photo-degenerated retina. MCO is reliably delivered to specific retinal cells via intravitreal injection of Adeno-Associated Virus, leading to significant improvement in visually guided behavior conducted using a radial-arm water maze. The number of error arms and time to reach platform significantly reduced after delivery of MCO. Notably, the improvement in visually guided behavior was observed even at light intensity levels orders of magnitude lower than that required for Channelrhodopsin-2 opsin. Biodistribution study using qPCR analysis showed negligible quantities of MCO-gene in different tissues of the treated mice. Safe virus-mediated MCO-delivery has potential for effective gene therapy of diverse retinal degenerations in patients.

10052-25, Session 8

Infrared light causes short- and long-term modulation of phrenic nerve activity

Gjinovefa Kola M.D., Emilie H. Lothet, Junqi Zhou, Case Western Reserve Univ. (United States); E. Duco Jansen, Vanderbilt Univ. (United States); Stephen J. Lewis, Hillel J. Chiel, Michael W. Jenkins, Case Western Reserve Univ. (United States)

Electrical stimulation of the proximal phrenic nerve connected to the brainstem increases ventilation and blood pressure. We hypothesized that infrared (IR) laser light would modulate phrenic nerve activity (PNA) without affecting the breathing pattern or autonomic activity.

In in situ arterially perfused preparations of rats (P15-P25, male, Sprague-Dawley. n=4), an optical fiber (200 μm) was positioned at the cut distal end of the phrenic nerve relative to the recording electrode. The IR light (? = 1464 or 1860 nm, 200 Hz frequency, 200 μs pulse duration) was applied for 20 s. Low radiant exposures facilitated PNA (both rhythmic (bursting) and tonic (steady) activities) whereas a slightly higher radiant exposure decreased and then blocked PNA. Further, if rhythmic PNA increased then it persisted for ~45-60min whereas if PNA was blocked, bursting resumed shortly after IR stimuli ceased. Autonomic nerve activity (vagal nerve activity and thoracic sympathetic chain ganglia) did not change.

Thus, PNA responds to direct IR stimulation of the phrenic nerve. The unexpected results include: 1) the sensitivity of PNA - opposite effects with a small change in IR strength and 2) the persistence of increased activity to IR that does not occur following electrical stimulation, but can be evoked by the repeated activation of PNA by serotonin or short hypoxic exposures.

We speculate that IR is a novel approach to evoke neuroplasticity and neuromodulation in motor nerve activity.

10052-26, Session 8

Modulation of pain by optogenetic stimulation of targeted neurons in specific brain regions

Sanjay Pradhan, Ki Yong Kwon, Samarendra K. Mohanty, Nanoscope Technologies, LLC (United States)

Neurons in different parts of the central nervous system (e.g. Thalamus, Anterior cingulate Cortex and spinal cord) receive projections from multiple ascending pain pathways. The deep brain regions are known to be involved in processing nociceptive information before transmitting the information to various parts of the cortex. Since electrical stimulation lacks the specificity to activate specific neurons (e.g. excitatory or inhibitory), use of electrical deep brain stimulation has not been effective in all chronic pain patients. Here, we demonstrate reliable modulation of pain by optogenetic stimulation of targeted neurons in specific brain regions in mouse model. Optogenetic stimulation of specific cortical or deep-brain regions significantly reduced response to particular types of painful stimuli (e.g., formalin injection). Our results underscore the distinct advantages of optogenetic stimulation for controlled modulation of pain, thus help in their control as well as dissection of pain pathways.

10052-27, Session 8

Safety and selectivity of infrared block for small-diameter axons

Emilie H. Lothet, Hui Lu, Case Western Reserve Univ. (United States); E. Duco Jansen, Vanderbilt Univ. (United States); Stephen J. Lewis, Michael W. Jenkins, Hillel J. Chiel, Case Western Reserve Univ. (United States)

Controlling subpopulations of unmyelinated axons within peripheral nerves would make it possible to treat a wide variety of clinical syndromes. Infrared (IR) light has been described as a promising modality for control of neural activity. Recent studies have shown that pulsed IR can inhibit neurons with high spatial specificity. We found that IR light (1860nm wavelength, 200Hz pulse, 200usec pulse width) can block nerves with axonal subpopulation selectivity, such that smaller-diameter axons with slower conduction velocities are more sensitive to IR light and are therefore more readily blocked than larger-diameter axons with faster conduction velocities.

To evaluate the clinical viability of an IR light as a blocking system, it is critical to determine the speed at which block can be initiated, how long the block can be maintained and damage thresholds. To determine these parameters, we used the unmyelinated Aplysia pleural-abdominal connectives. We found that, at a radiant exposure of 0.2 J/cm2, the shortest delay between the onset of infrared and block of the smaller axons was < 1 second. This delay increased as the radiant exposure was decreased. Selective block of small-diameter axons was maintained for up to 8 hours (N = 9). Because recovery was complete, this is probably not an upper limit for how long block could be maintained.

These results suggest that IR light can be used to block unmyelinated C fibers selectively, reversibly and for an extended period of time. IR may be a novel treatment option for significant clinical challenges such as chronic pain.

10052-22, Session PSun

Initial proof-of-concept of photoacoustic cell stimulation approach: preliminary in vitro study

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Localized, non-invasive cell stimulation method has been desired. Here we propose the initial proof-of-concept in vitro study of the photoacoustic cell stimulation approach, which is amenable to high-throughput screening applications. The proposed method is implemented as follows: 1) the localized excitation using the focused pulsed laser delivery on an absorptive material placed under the plate containing the cells, 2) cell stimulation by the photoacoustic pressure generated, and 3) fluorescence quantification of the membrane potential change over time. The preliminary proof-of-concept in vitro study is conducted with primary neurons isolated from mouse brain. The plate harboring primary neurons is situated above the absorptive rubber media which generates the photoacoustic pressure by the pulsed laser excitation. The experimental results show the feasibility of photoacoustic cell stimulation approach by indicating the significant membrane potential chance from the photoacoustically-stimulated primary neurons, which is comparable to that of the potassium chloride-administrated (KCl) control group. Otherwise, the sham control without any photoacoustic stimulation does not indicate any noticeable membrane potential change. We envisage that the proposed approach can allow broad strategies for non-invasive cell stimulation by using the photoacoustic contrasts situated at inside or outside of the body such as external absorptive materials or intravascularly-injected photoacoustic contrast particles.

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10053-85, Session PSun

Few-mode fiber optical coherence tomography for scattering characterization

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Optical tissue properties are directly accessible by imaging techniques and can be important diagnostic markers. Depending on the tissue microstructure, light is scattered in different directions. In this work, we present a few-mode fiber based swept source OCT system to extract information of the scattering properties depending on the angle of reflection in the tissue. Few-mode fibers transmit different modes depending on the angle of incidence into the fiber, delivering each mode through different paths. By using this characteristic, it is possible to encode in depth the information contained in different angles of incidence. The system was centered at 1310 nm with 140 nm bandwidth and the A-scan rate was 100 kHz. A low NA multimode step index fiber was used for modal-depth multiplexing in the sample arm. A calibration of the relation between angle of incidence in the fiber and intensity distribution of the fiber modes was performed, demonstrating a characteristic relation between the angle of incidence and the modal intensity distribution encoded in the A-scan. The system was tested for imaging ex vivo brain tissue, in order to investigate the modal detection in scattering tissue. While these imaging results were just preliminary, they may indicate that few-mode fiber OCT might be a simple extension towards tissue characterization.

10053-86, Session PSun

Phase-sensitive, complex-domain master-slave interferometry

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In this communication we report on the added functionality to optical coherence tomography (OCT) systems, enabled by complex-domain master-slave interferometry (cMSI): that of phase-sensitive measurements. Phase-sensitive optical coherence tomography (PhS-OCT) can be employed to characterize the flow properties of a biological tissue by using methods such as phase variance or Doppler OCT, and also to measure additional polarimetric properties of a sample. The cMSI method is an alternative to the conventional Fourier transform based method, however in our previous reports on MSI the phase had been discarded.

This is the first report where we investigate the phase-sensitive capabilities of the cMSI method. To this goal, we perform measurements on a flow phantom and carry out flow measurements for different flow rates. We compare the results obtained by both techniques, the conventional FT based spectral interferometry (applied to resampled data sets) and cMSI. The quality of the phase information retrieved using the cMSI method compares favorably to that obtained using the FT based conventional spectral domain interferometry.

10053-87, Session PSun

Graphics processor unit acceleration enables realtime endovascular doppler optical coherence tomography imaging

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Intravascular Optical Coherence Tomography (OCT) has previously been used in both bench-top and clinical environments to produce vascular images, and can be helpful in characterizing, among other pathologies, plaque build-up and impedances to normal blood flow. The raw data produced can also be processed to yield high-resolution blood velocity information, but this computation is expensive and has previously only been available a posteriori using post-processing software. Real-time Doppler OCT (DOCT) imaging has been demonstrated before in the skin and eye, but this capability has not been available to vascular surgeons.

Graphics Processing Units (GPUs) can be used to dramatically accelerate this type of distributed computation. In this paper we present a software package capable of real-time DOCT processing and circular image display using GPU acceleration designed to operate with catheter-based clinical OCT systems. This image data is overlayed onto structural images providing clinicians with live, high-resolution blood velocity information to complement anatomical data.

10053-88, Session PSun

Narrow linewidth of RF-switched SOA wavelength-swept laser by using modified switching signal pattern

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In this paper, we proposed a simple and novel method to obtain a narrow linewidth of RSS wavelength-swept laser using a reduced on/off time-ratio signal with a duty cycle of ~ 4% and demonstrated narrow linewidth RF-swiched SOA (RSS) wavelength-swept laser for deeper OCT imaging. The spectral linewidth becomes narrower about 10 times compared to the conventional RSS wavelength-swept laser. In the wavelength selection mechanism using the dispersive RSS cavity, the spectral linewidth is related to the on/off time-ratio of RF SOA switching signal. In case of conventional RSS wavelength-swept laser using the RF SOA switching signal with a duty cycle of 50 %, the only way to make a short SOA switching time and its resulting narrow spectral linewidth is to use a higher modulation frequency signal. However, the higher modulation frequency cause unwanted effect of the narrowed wavelength tuning region. Therefore, by using the reduced on/off time-ratio of RF SOA switching signal, we can alternatively improve the time-selectivity of wavelength output and success to make narrow the spectral linewidth without decreasing wavelength tuning region. In the reduced on/off time-ratio condition with a duty cycle of ~ 4 %, the spectral linewidth of RSS wavelength-swept laser is 0.027 nm, which is about 10 times narrower than the conventional result of 0.284 nm with a duty cycle of ~ 50 % condition.. This method can overcome a disadvantage for application of deeper OCT imaging due to the broad linewidth of the conventional RSS wavelength-swept laser.

10053-89, Session PSun

External amplification of OCT swept-sources for challenging applications: from 10 mW to more than 120 mW

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Since optical coherence tomography (OCT) is an interferometric imaging technique, it is sensitive enough to operate with extremely low levels of light. OCT is mostly used for bioimaging applications for which optical power is limited to a few milliwatts to avoid damaging the tissues. Commercial swept-sources currently provide enough power (tens of milliwatts) for these applications by relying mostly on the semiconductor optical amplifier technology. Nevertheless, more powerful swept-laser sources are required to bring the OCT knowledge from the bioimaging realm to other fields, like industrial monitoring and inspection. In industrial applications, surfaces can have strong specular reflection with low diffuse reflection, which represents quite a challenge when the surface is inspected at an angle. Practical configurations for online monitoring often require a large standoff distance for the measurement; the numerical aperture of the optics is consequently low, leading to less efficient light collection. The solution to these challenges is to increase optical power in the sample arm in order to ensure that more photons are collected to compensate for the low reflectivity. In this paper, we investigate three different setups to externally amplify the output of a commercial swept-source: a booster semiconductor optical amplifier (BOA), an erbium-doped fiber amplifier (EDFA) and a combination of both. The efficiency of the amplification is evaluated from the resulting optical spectrum and from the properties of point-spread functions measured with a custom-built swept-source OCT system. These external amplification setups allow the exploration of emerging OCT applications without the need to develop new hardware.

10053-90, Session PSun

Dispersion measurements in ocular media using a dual wavelength swept source optical coherence tomography system

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Optical coherence tomography (OCT) has proved to be a powerful tool for the detection of microstructure in tissue. Label free tissue differentiation on a micron scale is a promising and powerful technique for segmentation. This paper describes a technique using a dual wavelength swept source OCT system to image the eye. We measure the walk-off between interfaces in A-scans, taken at two different wavelengths, to calculate the group velocity dispersion parameter of each segment of the eye. We present measurements of the dispersion of the cornea and the aqueous humour in rat eyes.

The home build dual wavelength OCT setup uses two swept source lasers in grazing incidence configuration and gain chips centred at 1 and 1.3?m. Prior to the dispersion measurements in ocular media, calibration measurements were performed on glass, water and lipid. The calculated dispersion values for the calibration agreed closely with the known reference values for the materials and showed uncertainties of ±10%. To detect the surfaces of the ocular tissue and calculate a robust and stable dispersion value, a surface detection algorithm was developed using thresholding and polynomial fitting to the surface of interest. Dispersion calculations showed results for the aqueous humour of -37.48 ± 13.39 fs2/mm which is close to water with -38 fs2/mm at a wavelength of 1.2?m. Dispersion for the cornea was 4 times as high as water with -129.52 ± 24.32 fs2/mm.

10053-91, Session PSun

Improved ultrahigh-speed space-division multiplexing optical coherence tomography with integrated photonic devices

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Optical coherence tomography (OCT), an emerging biomedical imaging technology that enables micron?scale, cross?sectional, and three-dimensional (3D) imaging of biological tissues non-invasively, has been used in a wide range of clinical applications, including ophthalmology, cardiology, endoscopy, oncology, dermatology, and dentistry. In ophthalmology, high definition 3D scan of the patient's eye is greatly desired by ophthalmologists as it can assist them to track disease progression in the human retina. However, current commercial ophthalmic OCT systems are unable to provide high-quality, usable 3D scans to track any progression, due to severe motion artifacts caused by low imaging speed. Our group has proposed a space-division multiplexing OCT (SDM-OCT) technology that utilizes a parallel imaging scheme to achieve speed improvement over an order of magnitude as compared to current state-of-the-art commercial OCT systems. An effective 800,000 A-scans/s imaging speed was achieved in our first lab prototype, yielding the ability to visualize 3D structure of a fruit fly larva in less than 0.4 second. This pototype SDM-OCT system, however, requires extensive efforts to assemble fiber components and control optical delays between different channels by hand, which make it challenging for mass-reproduction. Recently, we have developed an integrated photonic chip for SDM-OCT to replace fiber-based components. Customed optical delays and spacing between each output beam of the chip can be precisely defined lithographically within sub-micron tolerances during the fabrication process. Development of integrated photonic devices will greatly lower the per-unit cost and facilitates broad dissemination of the SDM-OCT technology.

10053-92, Session PSun

High frame-rate en face optical coherence tomography system using KTN optical beam deflector

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There are three types of data acquisition and image processing by OCT: time domain (TD) OCT, Fourier domain (FD) / spectral domain (SD) OCT, and swept source (SS) OCT. SS-OCT offers the highest speed for data acquisition due to the wavelength-tuning speed of a swept source. Recently, MEMS vertical-cavity surface-emitting laser swept source provides sweeping speed of 60 kHz to 1 MHz. The Fourier domain mode-locked laser is considered to have the highest sweeping speed in the range of up to multi MHz.

The KTa1-xNbxO3 (KTN) crystal has a very large EO effect, which changes its refractive index when a voltage is applied and bends the path of a light beam in a new direction. The deflection effect of the KTN is caused by a non-uniform electric field generated by injected carriers, and exhibits a fast response of up to several hundred MHz and a fairly large beam deflection angle. Considering this performance, we propose a novel high speed en face OCT system that used a KTN optical deflector as the sample probe. In the imaging system, the fast scanning was performed at 200 kHz by the KTN optical beam deflector, while the slow scanning was performed at 800 Hz by the galvanometer mirror. As a preliminary experiment, we succeeded in obtaining en face OCT images of human fingerprint with a frame rate of 800 fps. This is the highest frame-rate obtained using TD en face OCT imaging. The 3D-OCT image of sweat gland was also obtained by our imaging system.

10053-93, Session PSun

Compact LED-based full-field optical coherence microscopy for high-resolution high-speed in vivo imaging

Jonas Ogien, Arnaud Dubois, Lab. Charles Fabry (France)

This work introduces a compact full-field optical coherence microscopy (FF-OCM) setup illuminated by a high brightness LED. The LED high radiance makes it possible to reach an image rate of 280 Hz with a 65 dB sensitivity, while maintaining a sub-micron axial resolution as in conventional thermally-illuminated FF-OCM. This high-speed implementation of FF-OCM makes it possible to perform in vivo imaging. A compact setup was specifically designed to pave the way for a portable system that will make it possible to image any region of interest in real-time. Human skin was imaged in vivo using this setup.

10053-94, Session PSun

Dependence on fiber Fabry-Pérot tunable filter characteristics in an all-fiber swept-wavelength laser for use in an optical coherence tomography system

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Optical coherence tomography (OCT) has become a useful and common diagnostic tool within the field of ophthalmology. Although presently a commercial technology, research continues in improving image quality and applying the imaging method to other tissue types. Swept-wavelength lasers based upon fiber ring cavities containing fiber Fabry-Pérot tunable filters (FFP-TF), as an intracavity element, provide swept-source optical coherence tomography (SS-OCT) systems with a robust and scalable platform. The FFP-TF can be fabricated within a large range of operating wavelengths, free spectral ranges (FSR), and finesses. To date, FFP-TF's have been fabricated at operating wavelengths from 400nm to 2.2?m, FSR's as large as 45THz, and finesses as high as 30000. The results in this paper will focus on presenting the capability of the FFP-TF as an intracavity element in producing swept-wavelength lasers sources and quantifying the tradeoff between coherence length and sweep range. We present results within a range of feasible operating conditions. Particular focus is given to the discovery of laser configurations that result in maximization of sweep range and/or power. A novel approach to the electronic drive of the PZT-based FFP-TF is also presented, which eliminates the need for the existence of a mechanical resonance of the optical device. This substantially increases the range of drive frequencies with which the filter can be driven and has a positive impact for both the short all-fiber laser cavity (presented in this paper) and long cavity FDML designs as well.

10053-95, Session PSun

Speckle variance full-field optical coherence microscopy for high-resolution microvasculature mapping

Jonas Ogien, Arnaud Dubois, Lab. Charles Fabry (France)

Speckle variance (SV) methods applied to conventional OCT images have shown very encouraging results in providing additional functional information about vasculature to the morphological information of OCT, at the same spatial resolution and without the need for any exogenous agent. Most of the time, the vascular network is contained within planes parallel to the surface of the tissues commonly imaged using OCT, therefore, en face imaging is adapted to visualize it. As conventional OCT provides vertical slices, a 3D volume of SV-OCT images must be obtained, before reslicing it to image the vasculature network in an en face view. Full-field optical coherence microscopy (FF-OCM) is an implementation of OCT combining full-field illumination and detection, where en face images are directly acquired using an area camera. SV methods implemented in an FF-OCM setup would be an easy way to map vasculature networks without the need for reconstruction, at a resolution higher than conventional OCT. Up to now, SV methods were not applicable to FF-OCM because of a too low acquisition speed. We recently built a high-speed, high-resolution FF-OCM setup using a high-brightness broadband LED, reaching an image rate of ~ 380 Hz. This work demonstrates the application of SV methods with this particular setup for imaging intralipid flowing into microcapillary tubes. Vasculature network imaging in mouse and human skin is demonstrated.

10053-96, Session PSun

Speckle variance optical coherence tomography using an SS-OCT system and an extended k-sampling clock

Reiko Yoshimura, Donghak Choi, Kitasato Univ. (Japan); Kohji Ohbayashi, Advanced Imaging Co., Ltd. (Japan)

In this work, we present an optical coherence angiography (OCA) system using swept-source optical coherence tomography (SS-OCT) with filtered k-sampling clock. We adopted the intensity-based Doppler variance (IBDV) method to obtain angiography images, which uses the complex OCT amplitude data. We developed an external k-sampling clock generator which provides enhanced depth ranges in OCT image with commercial frequency swept light source. In spite of the long coherence length, the k-sampling clock installed in a commercialized swept light source is set to observe the depth range of OCT image up to 5 mm. We intended to develop an external k-sampling clock for the commercialized light source and obtain OCT and OCA images in various depth ranges from 5 mm up to over 10 mm using the system.

10053-97, Session PSun

Optimization of data processing with the Akinetic swept-laser: algorithm to automatically adjust the A-scan synchronization delay

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The Akinetic swept-laser (Insight, Lafayette, USA) is an all-semiconductor compact and tunable laser source for optical coherence tomography (OCT). Tuning is performed by adjusting the refractive index at various locations in the device. Laser sweep with this source are composed of valid data sections interleaved with invalid data sections. The source provides a precise trigger at each wavelength sweep and a "data valid vector" (DVV) file which identifies the indices of the valid data points in a recorded interferogram. In order to identify valid data in real time during acquisition, a delay must be precisely adjusted between the trigger and the wavelength sweep. The source provides tools to optimize the delay, but they are not automated and require a sample with a single clean reflection. Optimizing the delay for DVV treatment with the Akinetic swept-laser becomes tedious when changing the scan parameters or when changing the interferometer configuration in a multi-purpose OCT system. A better tool is required for finding the delay correction that must be applied for DVV treatment. To do this, we developed a simple and robust algorithm integrated in our OCT data acquisition and treatment software. The algorithm automatically optimizes the delay that allows to accurately identify the valid data obtained with the Akinetic swept-laser for OCT. It can perform optimization from the laser spectrum or from the interferogram of a sample. It makes it easier to use the programmability features of the Akinetic swept-laser and to readjust the system when the interferometer configuration is modified.

10053-98, Session PSun

Improving contrast of swept source optical coherence tomography

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Swept source optical coherence tomography (SSOCT) can achieve real-time imaging in vivo due to its simple setup and high imaging speed. However the bio-tissue are mostly turbid and highly scattering, the incident light will attenuate rapidly, only 1-2mm microstructures of tissue can be detected clearly and the contrast of image will drop with depth sharply which restricted its development and application in clinical. In order to improve the depth imaging capability of SSOCT a dual channels method was presented in this paper. The interference signal of a SSOCT system was divided into two channels with a splitting ratio of 1:5. In the weaker channel the surface structure of tissue can be imaged at a high contrast, in the stronger channel in order to reconstruct deep structure of tissue the low frequencies signals are filtered, which ensure the signal intensity will not beyond the measurement range of analog to digital converter and the noise will keep at low level. After combining two signals in two channels a high contrast of image in both surface and deep of tissue will obtained. To demonstrate the feasibility of this method, a human finger were imaged. By comparing the OCT images reconstructed with and without this compensation method, a prominent improvement of OCT image contrast can be observed, especially in deep region.

10053-99, Session PSun

Miniature Mirau interferometry for swept-source OCT imaging with applications in dermatology

Christophe Gorecki, FEMTO-ST (France)

Commercially available interference microscopes often use two types of interferometric objectives, which are modifications of the Michelson interferometer: the Mirau and Linnik configurations. The specificity of Mirau interferometric objective is to place a reference mirror in the center of the objective lens, and interposing a semi-transparent plate between the objective lens and the specimen. Thus, such architecture is well suited to be vertically integrated by micromachining technologies. Here, MOEMS and MEMS components can be stacked using a multi-wafer vertical integration method to build the array-type MOEMS-based micro-interferometers. One of the instruments that can benefit from the significant improvement in equipment efficiency and quality of patient diagnosis made by incorporating MOEMS technology is the OCT microscopy dedied for the diagnosis of skin pathologies. In the frame of the European project VIAMOS, including 7 academic, research and industrial partners we proposed to develop a swept-source OCT (SS-OCT) microsystem based on spectrally tuned Mirau interferometry, into which a doublet of microlens matrices (4x4) and a wafer of movable reference mirrors are included, building the active Mirau interferometer. The incident light beam from a tunable swept-source operating at 840 nm (scan range 50 nm). The light is then collected by an array of microlenses and directed towards the skin sample. For each single-channel interferometer, the collected light passes through a thin beam-splitter plate that reflects half of it back to a moving reference mirror while the rest of light is transmitted towards the skin to be measured. The beams reflected by the sample and by the reference mirror interfere, generating an interference pattern directed by the microlens towards a high speed camera after a reflection on a cube beam-splitter. Such high speed camera combined to the actuated reference mirrors, lead to a phase shifting schema what enables a rapid measurement of the amplitudes and phases. The matrix of Mirau reference mirrors is integrated on top of an electrostatic vertical comb-drive actuator. The microsystem will acquire full 3D images at video rate with the lateral resolution of 6.2 μm and a penetration depth of 0.6 mm.

10053-100, Session PSun

Ultra-deep imaging of time-domain optical coherence tomography in highly scattering media

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A time-domain optical coherence tomography system based on measuring the reflection matrix of back-scattered light is proposed for extended imaging depth into highly scattering media. By applying a filtering operation to the measured reflection matrix, the back-scattered light with near-forward directions are preserved while the diffused multi-scattered light with random directions are mostly discarded. A singular value decomposition is then carried out to the filtered matrix for principal component analysis, to further separate the contribution of single scattered light from that of the residual multi-scattered light in the filtered matrix. The targets hidden inside the highly scattering media are then recovered according to the retrieved contribution of single scattered light. The resulting image resolution and contrast show that the near-forward propagating single scattered light, which is mostly discarded by the fiber-tip pinhole in conventional OCT, can be collected experimentally and separated computationally to increase the penetration depth of OCT after removing the multi-scattering components.

10053-101, Session PSun

Applying high dynamic range technique to recover the true depth information of optical coherence tomography

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Optical coherence tomography (OCT) is an advantageous non-invasive cross sectional imaging technology which has been widely applied in imaging of eyes, skins, vessels, cartilage and others. Due to the tomography system limitation, the pixel intensity responses are not uniformed to layers in the whole penetration range. In general, fewer and fewer photons can penetrate to the deeper sample and be reflected back to the OCT system for signal acquisition and image processing. Although increasing light source power can have more penetrated photons to enhance the pixel intensity at designated sample depth, the intensities of near surface portion are easily saturated which reduce image contrast and lose image details. Besides, the non-uniform intensity response is easy to mislead users on the tissue scattering distribution so as to the structures and layer thickness along the OCT image depth range. Herein, we report the use of special high dynamic range (HDR) technique to reconstruct an OCT true depth scattering image, correcting the conventional non-uniform intensity response problem at different sample depths. Through the use of a set of B-scan images captured with different integration times of the spectrometer and output powers of light source, we established the needed response functions of our OCT system at different sample depths. Using the response functions, multiple B-scans OCT images are fused together to reconstruct a single HDR image with a relatively uniform intensity response at different sample depths. We further used advanced tone mapping techniques to enhance the display of the HDR image in common low dynamic range monitors showing clear and correct image details in full depth range. The HDR technique can further enhance C-scan 3D images in the full image depth range.

10053-1, Session 1

Wavefront sensorless adaptive optics optical coherence tomography for multiphoton retinal imaging

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Two-photon excited fluorescence (TPEF) for in-vivo retinal imaging is an emerging tool for vision science. TPEF has multiple benefits in comparison to conventional confocal fluorescence scanning laser ophthalmoscopy for retinal imaging, including better axial resolution and the ability to use infrared excitation light for imaging the highly photosensitive tissue in the retina. TPEF is very sensitive to the focused spot size, which is enlarged by aberrations induced by the refractive elements of the mouse eye when imaging with a large numerical aperture. Our system begins with a femtosecond pulsed laser for two-photon excitation, which is also sufficiently spectrally broadband to allow for an optical coherence tomography (OCT) sub-system to guide aberration correction. The OCT system operated at 1 volumes/second with our custom GPU accelerated real-time processing. Our lens-based optical design features two deformable elements, one with large stroke for focus control on the retina and the other with multiple actuators for aberration correction. Our wavefront-sensorless adaptive optics (SAO) is driven by a modal search with a sharpness quality metric on the en-face OCT image of the selected retinal layer. After optimization, the speed was increased to 10 fps for TPEF imaging to allow for streaming and averaging ~200 frames per image. To demonstrate the system capabilities, we performed in-vivo retinal fluorescein angiography using TPEF. Our results demonstrate depth-resolved aberration correction with the SAO-OCT to increase the TPEF signal intensity. We also present TPEF at multiple vascular layers in the mouse retina alongside the volumetric OCT to localize the vessels.

10053-2, Session 1

In-vivo digital wavefront sensing using a 1060 nm swept source OCT

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DAO correction has already been successfully demonstrated for ex-vivo and in- vivo biological tissue and human retina OCT images . However, a high volume rate of more than 10 Hz is required for in-vivo OCT imaging to avoid any significant sample motion and maintain the phase stability necessary for any successful implementation of DAO. Hence, in-vivo implementation has been only shown using high speed enface time domain (TD) OCT and full field (FF) SS OCT achieving a corresponding 10 Hz and 180 Hz volume rate respectively, or recently with line field (LF) OCT . Digital wavefront sensing (DWS) is of great interest as it avoids the problems associated with Shack-Hartmann (S-H) sensor such as sensitivity to spurious back-reflections in the system and the limited dynamic range. In the present work, a fiber based point scanning SS OCT is used to scan a small lateral FOV of ~ 150x150 um? on the sample at an OCT volume rate of 17 Hz. It is shown that this small FOV can be used as a GS to detect optical aberrations introduced by the system and the sample using sub-aperture based DAO. The proof of principle is demonstrated using a micro-bead phantom sample. In-vivo aberration measurement with P-V value of ~1.7 waves, or equivalently a wavefront power error of ~0.2 dioptres, is shown for human photoreceptor OCT imaging.

10053-3, Session 1

Ultra-compact swept-source optical coherence tomography handheld probe with motorized focus adjustment

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Handheld optical coherence tomography (OCT) systems facilitate imaging of young children, bedridden subjects, and those with less stable fixation. Smaller and lighter OCT probes allow for more efficient imaging and reduced operator fatigue, which is critical for prolonged use in either the operating room or neonatal intensive care unit. In addition to size and weight, the imaging speed, image quality, field of view, resolution, and focus correction capability are critical parameters that determine the clinical utility of a handheld probe. Here, we describe an ultra-compact swept source (SS) OCT handheld probe weighing only 211 g (half the weight of the next lightest handheld SSOCT probe in the literature) with 20.1 μm lateral resolution, 7 μm axial resolution, 102 dB peak sensitivity, a 27° x 23° field of view, and motorized focus adjustment for refraction correction between -10 to +16 D. A 2D microelectromechanical systems (MEMS) scanner, a converging beam-at-scanner telescope configuration, and an optical design employing 6 different custom optics were used to minimize device size and weight while achieving diffraction limited performance throughout the system's field of view. Custom graphics processing unit (GPU)-accelerated software was used to provide real-time display of OCT B-scans and volumes. Retinal images were acquired from adult volunteers to demonstrate imaging performance.

10053-4, Session 1

Multimodal swept-source spectrally encoded scanning laser ophthalmoscopy and optical coherence tomography at 400 kHz

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Multimodal imaging systems that combine scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT) have demonstrated the utility of concurrent en face and volumetric imaging for aiming, eye tracking, bulk motion compensation, mosaicking, and contrast enhancement. However, this additional functionality trades off with increased system complexity and cost because both SLO and OCT generally require dedicated light sources, galvanometer scanners, relay and imaging optics, detectors, and control and digitization electronics. We previously demonstrated multimodal ophthalmic imaging using swept-source spectrally encoded SLO and OCT (SS-SESLO-OCT). Here, we present system enhancements and a new optical design that increase our SS-SESLO-OCT data throughput by >7x and field-of-view (FOV) by >4x. A 200 kHz 1060 nm Axsun swept-source was optically buffered to 400 kHz sweep-rate, and SESLO and OCT were simultaneously digitized on dual input channels of a 4 GS/s digitizer at 1.2 GS/s per channel using a custom k-clock. We show in vivo human imaging of the anterior segment out to the limbus and retinal fundus over a >40o FOV. In addition, nine overlapping volumetric SS-SESLO-OCT volumes were acquired under video-rate SESLO preview and guidance. In post-processing, all nine SESLO images and en face projections of the corresponding OCT volumes were mosaicked to show widefield multimodal fundus imaging with a >80o FOV. Concurrent multimodal SS-SESLO-OCT may have applications in clinical diagnostic imaging by enabling aiming, image registration, and multi-field mosaicking and benefit intraoperative imaging by allowing for real-time surgical feedback, instrument tracking, and overlays of computationally extracted image-based surrogate biomarkers of disease.

10053-5, Session 1

GPU accelerated optical coherence tomography angiography using strip-based registration

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High quality visualization of the retinal microvasculature can improve our understanding of the onset and development of retinal vascular diseases, which are a major cause of visual morbidity and are increasing in prevalence. Optical Coherence Tomography Angiography (OCT-A) images are acquired over multiple seconds and are particularly susceptible to motion artifacts, which are more prevalent when imaging patients with pathology whose ability to fixate is limited. The acquisition of multiple OCT-A images sequentially can be performed for the purpose of removing motion artifact and increasing the contrast of the vascular network through averaging. Due to the motion artifacts, a robust registration pipeline is needed before feature preserving image averaging can be performed.

In this report, we present a novel method for a GPU-accelerated pipeline for acquisition, processing, segmentation, and registration of multiple, sequentially acquired OCT-A images to correct for the motion artifacts in individual images for the purpose of averaging. High performance computing, blending CPU and GPU, was introduced to accelerate processing in order to provide high quality visualization of the retinal microvasculature and to enable a more accurate quantitative analysis in a clinically useful time frame. Specifically, image discontinuities caused by rapid micro-saccadic movements and image warping due to smoother reflex movements were corrected by strip-wise affine registration estimated using Scale Invariant Feature Transform (SIFT) keypoints and subsequent local similarity-based non-rigid registration. These techniques improve the image quality, increasing the value for clinical diagnosis and increasing the range of patients for whom high quality OCT-A images can be acquired.

10053-6, Session 1

Optimization method of superpixel analysis for retinal image of multifunctional JM-OCT

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Local statistics are widely utilized for quantification and image processing of OCT. For example, local mean is used to reduce speckle, local variation of polarization state (degree-of-polarization-uniformity (DOPU)) is used to visualize melanin. Conventionally, these statistics are calculated in a rectangle kernel whose size is uniform over the image. However, the fixed size and shape of the kernel result in a tradeoff between image sharpness and statistical accuracy. Superpixel is a cluster of pixels which is generated by grouping image pixels based on the spatial proximity and similarity of signal values. Superpixels have variant size and flexible shapes which preserve the tissue structure. Here we demonstrate a new superpixel method which is tailored for multifunctional Jones matrix OCT (JM-OCT). This new method forms the superpixels by clustering image pixels in a 6-dimensional (6-D) feature space (spatial two dimensions and four dimensions of optical features). All image pixels were clustered based on their spatial proximity and optical feature similarity. The optical features are scattering, OCT-A, birefringence and DOPU. The method is applied to retinal OCT. Generated superpixels preserve the tissue structures such as retinal layers, sclera, vessels, and retinal pigment epithelium. Hence, superpixel can be utilized as a local statistics kernel which would be more suitable than a uniform rectangle kernel. Superpixelized image also can be used for further image processing and analysis. Since it reduces the number of pixels to be analyzed, it reduce the computational cost of such image processing.

10053-7, Session 2

Ultrahigh-resolution capsule OCT at 800 nm

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We present an ultrahigh-resolution, distal-scanning OCT capsule operating at 800 nm targeted towards gastrointestinal tract imaging. Among many others, one significant challenge in the 800-nm OCT capsule technology is the severe chromatic aberration in the imaging optics of the capsule. By combining commercial miniature lenses and customized diffractive lens, the achromatic focal shift was essentially eliminated (i.e. down to ~1 μm) over a 3dB spectral bandwidth of ~150 nm centered around 825 nm. We have achieved an axial resolution ~2.7 μm. Initial proof-of-concept experiments with ex vivo pig esophagus demonstrated the excellent imaging performance of this 800-nm OCT capsule.

10053-8, Session 2

Ultrahigh resolution optical coherence elastography combined with a rigid micro-endoscope

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The mechanical forces that living cells experience represent an important framework in the determination of a range of intricate cellular functions and processes. Current insight into cell mechanics is typically provided by in vitro measurement systems; for example, atomic force microscopy (AFM) measurements are performed on cells in culture or, at best, on freshly excised tissue. Optical techniques, such as Brillouin microscopy and optical elastography, have been used for ex vivo and in situ imaging, recently achieving cellular-scale resolution. The utility of these techniques in cell mechanics lies in quick, three-dimensional and label-free mechanical imaging. Translation of these techniques toward minimally invasive in vivo imaging would provide unprecedented capabilities in tissue characterization. Here, we take the first steps along this path by incorporating a gradient-index micro-endoscope into an ultrahigh resolution optical elastography system. Using this endoscope, a lateral resolution of 2 μm is preserved over an extended depth-of-field of 80 μm, achieved by Bessel beam illumination. We demonstrate this combined system by imaging stiffness of a silicone phantom containing stiff inclusions and a freshly excised murine liver tissue. Additionally, we test this system on murine ribs in situ. We show that our approach can provide high quality extended depth-of-field images through an endoscope and has the potential to measure cell mechanics deep in tissue. Eventually, we believe this tool will be capable of studying biological processes and disease progression in vivo.

10053-9, Session 2

Design of tethered capsule endomicroscopy devices for Barrett's esophagus screening

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Optical coherence tomography (OCT) [ ] is an imaging technology that provides depth-resolved images by using interferometry to measure the optical delay of backscattered light from a sample. This technology has been shown in prior studies to be capable of accurately diagnosing Barrett's Esophagus (BE), a metaplastic change that conveys an increased risk of developing esophageal adenocarcinoma (EAC). Tethered capsule endomicroscopy (TCE) using OCT is a technology developed in our lab where a tethered, opto-mechanical pill is swallowed and obtains 10 μm resolution cross-sectional OCT images of the entire esophageal wall as the device traverses the esophagus via peristalsis.

Our first generation TCE device used a rotary junction (RJ) and a driveshaft to convey torque that rotated optics within the capsule to scan the beam along the esophageal wall. The cost of this driveshaft-based device was high, potentially hindering widespread adoption of this technology for screening. In this work, we have developed a next-generation TCE device that is significantly less expensive in both fixed and disposable costs while producing better and more quantitative image data. In the new design, we replaced the expensive RJ and driveshaft by an inexpensive cell-phone micro-motor costing less than $10. Implementing this second-generation device was challenging because inexpensive cell-phone motors are not designed to reliably attain constant velocity of rotation. This issue was mitigated through a combination of pulse-width drive modulation, appropriate loading of the motor, and by using image-based control-loop feedback. In this paper, we will describe these technical solutions in detail and present human imaging data obtained using our inexpensive cell-phone motor-based TCE device. Based on our results with this new technology, we believe that the cost of TCE devices can be lowered to the level that would be required to implement widespread OCT screening for BE.

10053-10, Session 2

Design and optimization of a miniaturized imaging probe for simultaneous endomicroscopy and optical coherence tomography

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We present a novel bimodal endoscopic imaging probe design that can simultaneously provide full-field white light video microscopy and confocal optical coherence tomography (OCT). The two modalities rely on spectrally-separated optical paths that run partially in parallel through a micro-optical bench system, which has dimensions of only 2 x 3 x 13 mm3 and is realized via standard silicon micromachining techniques. This approach avoids the inherent drawbacks of using a fiber bundle for OCT, such as low light throughput, multi-modal coupling and poor resolution for a given field of view. With a numerical aperture of 0.068, the video modality has a resolution and field of view of 6.2 ?m and 1 mm in diameter, respectively. The OCT modality is optimized for 1.3 μm center wavelength, and works in a scanning arrangement with a NA of 0.022. An integrated fiber scanner provides two dimensional depth probing with the same field of view as the video image with a lateral resolution of 26 ?m. To achieve this, a combination of silicon-based MEMS technology and polyimide-based flexible interconnects are used to realize a highly integrated piezoelectric fiber scanner with an outer diameter of 0.9 mm and a length of 9 mm. Through its compact footprint and enhanced functionality, the probe is designed to provide depth-resolved imaging capability for existing laparoscopes and represents a major step towards a new class of multi-modal endoscopic imaging probes.

10053-11, Session 2

Reconstruction of depth-resolved birefringence axis for imaging through rotating catheters

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Muscle and collagen fibers exhibit tissue birefringence and offer intrinsic contrast when imaged with polarization sensitive optical coherence tomography (PS-OCT). The orientation of the fast axis of the birefringent tissue would further enable to differentiate between tissue layers with distinct orientations and provide additional insight into their micro-structural organization. However, reconstructing the depth-resolved optic axis orientation is challenging, since all preceding tissue layers influence the apparent axis of rotation of the measured polarization states. In catheter-based imaging, the polarization state incident on the tissue is in addition unknown and changing with the rotation of the imaging probe. Here, we demonstrate depth-resolved optic axis imaging with catheter-based PS-OCT, using a conventional inter-A-line modulated PS-OCT system and without requiring any additional calibration signals. We employed a symmetry constraint, imposed by using identical illumination and detection optics, which simplifies the analysis of the signal from the catheter tip to estimate the transmission through the system components and the rotating catheter. Lastly, we used the intrinsic birefringence of the catheter sheath to orient the measured polarization states in absolute coordinates. This correction enabled reconstruction of the depth-resolved optic axis orientation of human coronary arteries, and revealed a distinct birefringence orientation in the individual layers of the vessel wall. Catheter-based imaging of collagen and muscle fiber orientation could offer insight into the pathogenesis and complications of atherosclerosis and the remodeling of the airway smooth muscle layer associated with asthma.

10053-12, Session 2

Anastigmatic needle probe for high-speed interstitial OCT imaging

Scott Wu Yuan, Xingde Li, Johns Hopkins Univ. (United States)

We report an anastigmatic needle probe made with fiber-optic ball lens for high-speed circumferential interstitial OCT imaging. The anastigmatic design affords a high transverse resolution of ~11.9 μm. The improved mechanical design enables a robust circumferential scanning speed up to ~26.8 frames per second. The miniaturized needle probe has an outer diameter of ~620 μm including the encasing metal guard and glass microcapillary. The performance of the anastigmatic OCT needle was demonstrated by imaging rat belly tissues and rat liver ex vivo with a 1300-nm swept-source OCT (SSOCT) system. The preliminary results suggest the potential of the needle probe for minimally invasive interstitial imaging and image-guided biopsy.

10053-13, Session 3

High-speed 4d intrasurgical microscope integrated optical coherence tomography at 800 kHz line rate using temporal spectral splitting

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The en face operating stereomicroscope offers limited depth perception and ophthalmic surgeons must often rely on stereopsis and instrument shadowing to estimate motion in the axial dimension. Recent research and commercial microscope-integrated optical coherence tomography (MIOCT) systems have allowed OCT of live surgery, but these were restricted to real-time cross-sectional (B-scan) imaging which captures limited information about maneuvers that extend over 3D space. We recently reported on a four dimensional (4D: 3D imaging over time) MIOCT and HUD system with real-time volumetric rendering for human ophthalmic surgery, but this 100 kHz OCT system was restricted to 3.3 volumes/sec to achieve sufficient lateral sampling over a 5x5 mm field of view (FOV). In this work, we present a high-speed 4D MIOCT (HS 4D MIOCT) system for volumetric imaging at 800 kHz A-scan rate. The proposed system employs a temporal spectral splitting (TSS) technique in which the spectrum of a buffered 400 kHz OCT system is windowed into sub-spectra to yield A-scans with reduced axial resolution but at a doubled A-scan rate of 800 kHz. The trade-offs of TSS for B-scan and volumetric retinal imaging were characterized in healthy adult volunteers. In addition, porcine eye surgical manipulations were imaged with HS 4D MIOCT imaging at 10.85 volumes/sec with 400x96x340 (X,Y,Z) usable voxels over a 5x5 mm lateral FOV. HS 4D MIOCT was capable of imaging subtle volumetric tissue manipulations with high temporal and spatial resolution using ANSI-limited optical power and is readily translatable to the human operating suite.

10053-14, Session 3

Visualizing microvascular flow variation in OCTA using variable interscan time analysis (VISTA)

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OCT angiography (OCTA) has recently garnered immense interest in clinical ophthalmology, permitting ocular vasculature to be viewed in exquisite detail, in vivo, and without the injection of exogenous dyes. However, commercial OCTA systems provide little information about actual erythrocyte speeds; instead, OCTA is typically used to visualize the presence and/or absence of vasculature. This is an important limitation because in many ocular diseases, including diabetic retinopathy (DR) and age-related macular degeneration (AMD), alterations in blood flow, but not necessarily only the presence or absence of vasculature, are thought to be important in understanding pathogenesis. To address this limitation, we have developed an algorithm, variable interscan time analysis (VISTA), which is capable of resolving different erythrocyte speeds. VISTA works by acquiring >2 repeated B-scans, and then computing multiple OCTA signals corresponding to different effective interscan times. The OCTA signals corresponding to different effective interscan times contain independent information about erythrocyte speed. In this study we provide a theoretical overview of VISTA, and investigate the utility of VISTA in studying blood flow alterations in ocular disease. OCTA-VISTA images of eyes with choroidal neovascularization, geographic atrophy, and diabetic retinopathy are presented.

10053-15, Session 3

Imaging of physiological responses to photostimulation in human photoreceptors with full-field swept-source OCT

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The non-invasive measurement of cellular physiological responses to photostimulation in living retina may have significant clinical value and give new insight into the vision process. Optical coherence tomography (OCT) has been reported to detect suitable intrinsic optical signals (IOS) in retinal photoreceptor layers upon their stimulation. Commonly, changes in backscattering intensity were observed ex vivo and immobilized animals in vivo. However, in humans measurements were time-consuming and cumbersome. Promising results were achieved when observing phase signals to detect intrinsic optical signals. But to achieve sufficient phase stability to image an entire area of photoreceptors turned out to be challenging. Here, we report full-field swept-source OCT to be sufficiently stable to detect the phase signals after projecting a stimulation image onto the living human retina. We extracted time-courses and signal dependencies from the measured datasets. For long stimuli, we were even able to assign responses to single cones. This functional imaging of photoreceptor activity could potentially be used to detect loss of photoreceptor function prior to visible morphological changes, which is associated with numerous retinal diseases.

10053-16, Session 3

Structural and functional human retinal imaging with a visible light OCT ophthalmoscope

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Visible light is absorbed by intrinsic chromophores such as photopigment, melanin, and hemoglobin, and scattered by subcellular structures, all of which are potential retinal disease biomarkers. Recently, high-resolution quantitative measurement and mapping of hemoglobin concentrations was demonstrated using visible light Optical Coherence Tomography (OCT). Yet, most high-resolution visible light OCT systems adopt free-space, or bulk, optical setups, which could limit clinical applications. Here, the construction of a multi-functional fiber-optic OCT system for human retinal imaging with <2.5 micron axial resolution is described. A detailed noise characterization of two supercontinuum light sources with differing pulse repetition rates is presented. The higher repetition rate, lower noise, source is found to enable a sensitivity of 87 dB with 0.1 mW incident power at the cornea and a 98 microsecond exposure time. Using a broadband, asymmetric, fused single-mode fiber coupler designed for visible wavelengths, the sample arm is integrated into an ophthalmoscope platform, rendering it portable and suitable for clinical use. In vivo anatomical, Doppler, and spectroscopic imaging of the human retina is further demonstrated using a single oversampled B-scan. For spectroscopic fitting of oxyhemoglobin (HbO2) and deoxyhemoglobin (Hb) content in the retinal vessels, a noise bias-corrected absorbance spectrum is estimated using a sliding short-time Fourier transform of the complex OCT signal and fit using a model of light absorption and scattering. This yielded path length (L) times molar concentration, LCHbO2 and LCHb. Based on these results, we conclude that high-resolution visible light OCT has potential for depth-resolved functional imaging of the eye.

10053-17, Session 3

Image-guided feedback for ophthalmic microsurgery using multimodal intraoperative swept-source spectrally encoded scanning laser ophthalmoscopy and optical coherence tomography

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Surgical interventions for ocular diseases involve manipulations of semi-transparent structures in the eye, but limited visualization of these tissue layers remains a critical barrier to developing novel surgical techniques and improving clinical outcomes. We addressed limitations in image-guided ophthalmic microsurgery by using microscope-integrated multimodal intraoperative swept-source spectrally encoded scanning laser ophthalmoscopy and optical coherence tomography (iSS-SESLO-OCT). We previously demonstrated in vivo human ophthalmic imaging using SS-SESLO-OCT, which enabled simultaneous acquisition of en face SESLO images with every OCT cross-section. Here, we integrated our new 400 kHz iSS-SESLO-OCT, which used a buffered Axsun 1060 nm swept-source, with a surgical microscope and TrueVision stereoscopic viewing system to provide image-based feedback. In vivo human imaging performance was demonstrated on a healthy volunteer, and simulated surgical maneuvers were performed in ex vivo porcine eyes. Densely-sampled static volumes and volumes subsampled at 10 volumes-per-second were used to visualize tissue deformations and surgical dynamics during corneal sweeps, compressions, and dissections, and retinal sweeps, compressions, and elevations. En face SESLO images enabled orientation and co-registration with the widefield surgical microscope view while OCT imaging enabled depth-resolved visualization of surgical instrument positions relative to anatomic structures-of-interest. TrueVision heads-up display allowed for side-by-side viewing of the surgical field with SESLO and OCT previews for real-time feedback, and we demonstrated novel integrated segmentation overlays for augmented-reality surgical guidance. Integration of these complementary imaging modalities may benefit surgical outcomes by enabling real-time intraoperative visualization of surgical plans, instrument positions, tissue deformations, and image-based surrogate biomarkers correlated with completion of surgical goals.

10053-18, Session 3

Investigation of the benefit of adaptive optics optical coherence tomography angiography for imaging the human retina

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In this work we investigate the benefits of using optical coherence tomography angiography (OCTA) in combination with adaptive optics (AO) technology. It has been demonstrated that the contrast of vessels and small capillaries can be greatly enhanced by the use of OCTA. Moreover, small capillaries that are below the transverse resolution of the ophthalmic instrument can be detected. This opens unique opportunities for diagnosing retinal diseases. However, there are some limitations of this technology such as shadowing artifacts caused by overlying vasculature or the inability to determine the true extension of a vessel. Thus, the evaluation of the vascular structure and density can be misleading. To overcome these limitations we applied the OCT angiography technique to images recorded with AO-OCT. Due to the higher collection efficiency of AO-OCT in comparison with standard OCT an increased intensity contrast of vasculature can be seen. Using AO-OCTA the contrast of the vasculature to the surrounding static tissue is further increased. The improved transverse resolution and the reduced depth of focus of the AO-OCT greatly reduce shadowing artifacts allowing for a correct differentiation and segmentation of different vascular layers of the inner retina. The method is investigated in healthy volunteers and in patients with diabetic retinopathy.

10053-19, Session 3

Retinal imaging with adaptive optics full-field OCT

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We demonstrated a simple adaptive optics full-filed OCT (FFOCT) with a transmissive liquid crystal spatial light modulator (LCSLM) as wavefront corrector that is used without strict plane conjugation for low order aberrations corrections. With an USAF resolution target, we validated experimentally that FFOCT resolution is almost independent of aberrations and only reduce the signal level due to the use of a spatially incoherent illumination. A signal based sensorless algorithm was thus applied for wavefront distortion compensation. Image quality improvements by the wavefront sensorless control of the LCSLM were evaluated on USAF resolution target for non-conjugated LCSLM-induced random aberration correction and also on Ficus leaves as well as fixed mouse brain tissue slice for sample self-induced aberration correction. By replacing the FFOCT sample arm objective with an artificial eye used to train ophthalmologists, adaptive optics retinal imaging was also achieved with improved signal to noise ratio. In vivo experiments using a liquid lens designed for focus and astigmatism correction are underway.

10053-20, Session 3

Comparison of different scanning optics geometries for anterior segment PS-OCT imaging

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Standard telecentric scanning optics for imaging the human cornea suffer from low signal intensities in areas where the beam has a large inclination to the corneal surface. We demonstrate a different scanning optics design for PS-OCT which results in imaging beams that are nearly orthogonal to the entire corneal surface. With this method good signal quality can be achieved for the entire cornea. Birefringence effects introduced by the cornea depend on the angle of the imaging beam to fibril orientation of the cornea. These fibrils are organized in lamellae parallel to the surface. Each of those lamellae contains highly organized parallel collagen fibrils which subtend large angles with fibrils in adjacent lamellae. These fibrils introduce form-birefringence which can be detected with PS-OCT. In addition, there are preferentially aligned, reinforcing collagen fibrils with a larger diameter, which are thought to help maintain the structural integrity of the cornea and adjacent sclera. Using the alternative scanning geometry, the influence of the corneal shape on the corneal birefringence can be greatly reduced which allows for the visualization of different fibril structures within the cornea. Especially the effects of preferentially aligned collagen fibrils on the polarization state is investigated. The results of our in vivo measurements are in good agreement with the results obtained from ex vivo X- ray scattering experiments.

10053-21, Session 4

Assessment of vascularization and myelination following peripheral nerve repair using angiographic and polarization sensitive optical coherence tomography

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A severe traumatic injury to a peripheral nerve often requires surgical graft repair. However, functional recovery after these surgical repairs is often unsatisfactory. To improve interventional procedures, it is important to understand the regeneration of the nerve grafts. The rodent sciatic nerve is commonly used to investigate these parameters. However, the ability to longitudinally assess the reinnervation of injured nerves are limited, and to our knowledge, no methods currently exist to investigate the timing of the revascularization in functional recovery.

In this work, we describe the development and use of angiographic and polarization-sensitive (PS) optical coherence tomography (OCT) to visualize the vascularization, demyelination and remyelination of peripheral nerve healing after crush and transection injuries, and across a variety of graft repair methods. A microscope was customized to provide 3.6 cm fields of view along the nerve axis with a capability to track the nerve height to maintain the nerve within the focal plane. Motion artifact rejection was implemented in the angiography algorithm to reduce degradation by bulk respiratory motion in the hindlimb site. Vectorial birefringence imaging methods were developed to significantly enhance the accuracy of myelination measurements and to discriminate birefringent contributions from the myelin and epineurium. These results demonstrate that the OCT platform has the potential to reveal new insights in preclinical studies and may ultimately provide a means for clinical intra-surgical assessment of peripheral nerve function.

10053-22, Session 4

Imaging ischemic strokes in rodents using visible-light optical coherence tomography

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Monitoring cortical hemodynamic response after ischemic stroke (IS) is essential for understanding the pathophysiological mechanisms behind IS-induced neuron loss. Functional optical coherence tomography (OCT) is an emerging technology that can fulfill the requirement, providing label-free, high-resolution 3D images of cerebral hemodynamics.

Unfortunately, strong tissue scattering pose a significant challenge for existing OCT oximetry techniques, as they either ignore the effect or compensate it numerically. Here we developed a novel dual-depth sampling and normalization strategy using visible-light OCT (vis-OCT) angiograms that can provide robust and precise sO2 estimations within cerebral circulation. The related theoretical formulation were established, and its implication and limitations were discussed.

We monitored mouse cortical hemodynamics using the newly-developed method. Focal ischemic stroke was induced through photothrombosis. The analysis on pre- and post-IS vis-OCT images revealed both vascular morphology and oxygenation altered substantially after the occlusion. First, the ischemic core could be clearly identified as angiographic intensity fell below the detection limit. In addition, vessel dilation presented universally in the penumbra region. Notably for pial arteriles, the percentage of increase demonstrated inverse relationship with their pre-occlusion, pre-dilation dimeter.

Vis-OCT oxygenation maps on intact cortex revealed spatial sO2 variations within pial vessels. Specifically, sO2 in arterioles decreased as it bifurcated and plunged into deeper tissue. Similarly, venous sO2 was higher in the larger, more superficial pial brunches. However, such difference was no longer appreciable after photothrombosis. Averaged arteriole sO2 dropped to 64% – 67% in the penumbra region.

10053-23, Session 4

Detection of cortical optical changes during seizure activity using optical coherence tomography

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Electrophysiology has remained the gold standard of neural activity detection but its resolution and high susceptibility to noise and motion artifact limit its efficiency. Imaging techniques, including fMRI, intrinsic optical imaging, and diffuse optical imaging, have been used to detect neural activity, but rely on indirect measurements such as changes in blood flow. Fluorescence-based techniques, including genetically encoded indicators, are powerful techniques, but require introduction of an exogenous fluorophore. A more direct optical imaging technique is optical coherence tomography (OCT), a label-free, high resolution, and minimally invasive imaging technique that can produce depth-resolved cross-sectional and 3D images. In this study, we sought to examine non-vascular depth-dependent optical changes directly related to neural activity. We used an OCT system centered at 1310 nm to search for changes in an ex vivo

brain slice preparation and an in vivo model during 4-AP induced seizure onset and propagation with respect to electrical recording. By utilizing Doppler OCT and the depth-dependency of the attenuation coefficient, we demonstrate the ability to locate and remove the optical effects of vasculature within the upper regions of the cortex from in vivo attenuation calculations. The results of this study show a non-vascular decrease in intensity and attenuation in ex vivo and in vivo seizure models, respectively. Regions exhibiting decreased optical changes show significant temporal correlation to regions of increased electrical activity during seizure. This study allows for a thorough and biologically relevant analysis of the optical signature of seizure activity both ex vivo and in vivo using OCT.

10053-24, Session 4

Scatter labeled imaging of microvasculature in embryos using optical coherence tomography

Yehe Liu, Shi Gu, Michiko Watanabe, Andrew M. Rollins, Michael W. Jenkins, Case Western Reserve Univ. (United States)

Abnormal coronary development causes various health problems. However, coronary development remains one of the highly neglected areas in developmental cardiology due to limited technology. Currently, there is not a robust method available to map the microvasculature throughout the entire embryonic heart in 3D. This is a challenging task because it requires both micron level resolution over a large field of view and sufficient imaging depth. Speckle-variance optical coherence tomography (OCT) has reasonable resolution for coronary vessel mapping, but limited penetration depth and sensitivity to bulk motion made it impossible to apply this method to late-stage beating hearts. Some success has been achieved with coronary dye perfusion, but smaller vessels are not efficiently stained and penetration depth is still an issue. To address this problem, we present an OCT imaging procedure using optical clearing and a contrast agent (titanium dioxide) that enables 3D mapping of the coronary microvasculature in developing embryonic hearts. In brief, the hearts of stage 36 quail embryos were perfused with a low viscosity mixture of polyvinyl acetate (PVA) and titanium dioxide through the aorta using micropipette injection. After perfusion, the viscosity of the solution was increased by crosslinking the PVA polymer chains with borate ions. The tissue was then optically cleared. The titanium dioxide particles remaining in the coronaries provided a strong OCT signal, while the rest of the cardiac structures became relatively transparent. Using this technique, we are able to investigate coronary morphologies in different disease models.

10053-25, Session 4

3D characterization of EMT cell density in developing cardiac cushions with optical coherence tomography

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Congenital heart defects (CHDs) are the most common birth defect, affecting between 4 and 75 per 1,000 live births depending on the inclusion criteria. Many of these defects can be traced to defects of cardiac cushions, critical structures during development that serve as precursors to many structures in the mature heart, including the atrial and ventricular septa, and all four sets of cardiac valves. Epithelial-mesenchymal transition (EMT) is the process through which cardiac cushions become populated with cells. Altered cushion size or altered cushion cell density has been linked to many forms of CHDs, however, quantitation of cell density in the complex 3D cushion structure poses a significant challenge to conventional histology. Optical coherence tomography (OCT) is a technique capable of 3D imaging of the developing heart, but typically lacks the resolution to differentiate individual cells. Our goal is to develop an algorithm to quantitatively characterize the density of cells in the developing cushion using 3D OCT imaging. First, in a heart volume, the atrioventricular (AV) cushions were manually segmented. Next, all voxel values in the region of interest were pooled together to generate a histogram. Finally, two populations of voxels were classified using either K-means classification, or a Gaussian mixture model (GMM). The voxel population with higher values represents cells in the cushion. To test the algorithm, we imaged and evaluated avian embryonic hearts at looping stages. As expected, our result suggested that the cell density increases with developmental stages. We validated the technique against scoring by expert readers.

10053-26, Session 4

Non-invasive red light optogenetic pacing and optical coherence microscopy (OCM) imaging for Drosophila melanogaster

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Cardiac pacing could be a powerful tool for investigating mammalian cardiac electrical conduction systems as well as for treatment of certain cardiac pathologies. However, traditional electrical pacing using pacemaker requires an invasive surgical procedure. Electrical currents from the implanted electrodes can also cause damage to heart tissue, further restricting its utility. Optogenetic pacing has been developed as a promising, non-invasive alternative to electrical stimulation for controlling animal heart rhythms. It induces heart contractions by shining pulsed light on transgene-generated microbial opsins, which in turn activate the light gated ion channels in animal hearts. However, commonly used opsins in optogenetic pacing, such as channelrhodopsin-2 (ChR2), require short light wavelength stimulation (475 nm), which is strongly absorbed and scattered by tissue. Here, we performed optogenetic pacing by expression of recently engineered red-shifted microbial opsins, ReaChR and CsChrimson, in a well-established animal model, Drosophila melanogaster, using the 617 nm stimulation light pulses. The OCM technique enables non-invasive optical imaging of animal hearts with high speed and ultrahigh axial and transverse resolutions. We integrated a customized OCM system with the optical stimulation system to monitor the optogenetic pacing noninvasively. The use of red-sifted opsins enabled deeper penetration of simulating light at lower power, which is promising for applications of optogenetic pacing in mammalian cardiac pathology studies or clinical treatments in the future.

10053-27, Session 4

Examining the prevention of alcohol-induced congenital defects using optical coherence tomography

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Nearly 2 million women in the United States alone are at risk for an alcohol-exposed pregnancy, including more than 600,000 who binge drink. Even low levels of prenatal alcohol exposure (PAE) can lead to a variety of birth defects, including craniofacial and neurodevelopmental defects, as well as increased risk of miscarriages and stillbirths. Studies

have also shown an interaction between drinking while pregnant and an increase in congenital heart defects (CHD), including atrioventricular septal defects and other malformations. We have previously established a quail model of PAE, modeling a single binge drinking episode in the third week of a woman's pregnancy. Using optical coherence tomography (OCT), we quantified intraventricular septum thickness, great vessel diameters, and atrioventricular valve volumes. Early-stage ethanol-exposed embryos had smaller cardiac cushions (valve precursors) and increased retrograde flow, while late-stage embryos presented with gross head/body defects, and exhibited smaller atrio-ventricular (AV) valves, interventricular septum, and aortic vessels. We previously showed that supplementation with the methyl donor betaine reduced gross defects, improved survival rates, and prevented cardiac defects. Here we show that these preventative effects are also observed with folate (another methyl donor) supplementation. Folate also appears to normalize retrograde flow levels which are elevated by ethanol exposure. Finally, preliminary findings have shown that glutathione, a crucial antioxidant, is noticeably effective at improving survival rates and minimizing gross defects in ethanol-exposed embryos. Current investigations will examine the impact of glutathione supplementation on PAE-related CHDs.

10053-28, Session 4

Characterizing 3D morphology of multicellular tumor spheroids using optical coherence tomography

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There is strong evidence that the morphological parameters of multicellular tumor spheroids (MCTS), particularly size, sphericity, and growth pattern, play a role in their cytochemical responses. Because tumor spheroids accurately represent the three-dimensional (3D) structure of in vivo tumors, they may also mimic in vivo cytochemical responses, thus lending them relevance to cancer research. Knowledge of MCTS attributes, including oxygen and nutrient gradients, hypoxia resistance, and drug response, assist specialists seeking the most efficient ways to treat cancer. Structural information on tumor spheroids can provide insight into these attributes, and become a valuable asset for treatment in vivo. Currently, high-resolution bioimaging modalities, most notably bright field imaging, phase contrast imaging, fluorescent microscopy, and confocal imaging, are being employed for this purpose. However, these modalities lack sufficient penetration depth to resolve the entire geometry of large spheroids (>200um). In response to this deficiency, we propose a potential high-throughput imaging platform using optical coherence tomography (OCT) to quantify MCTS morphology. OCT's high resolution and depth penetration allow us to obtain complete, high-detailed, 3D tumor reconstructions with accurate diameter measurements. Furthermore, a computer-based voxel counting method is used to quantify tumor volume, which is significantly more accurate than the estimations required by 2D-projection modalities. Thus, this imaging platform provides one of the most complete and robust evaluations of tumor spheroid morphology, and shows great potential for contribution to the study of cancer treatment and drug discovery.

10053-102, Session PMon

Bias correction of von Neumann entropy for polarization-sensitive OCT

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It is known that the randomness of the polarization property is useful for classifying biological tissues. For example, degree of polarization uniformity (DOPU) has been used in the segmentation of retinal pigment epithelium that has polarization scrambling effect of melanin. DOPU is defined as a uniformity of the state of polarized light. Although it is known to be useful, it does not show a uniformity of the polarization property of the sample. Recently, we suggested von Neumann entropy as a naturally scalable parameter to characterize the randomness of Jones matrix. However, a common issue of these parameters is that the random polarization property of the sample cannot be distinguished from the random noise, which results in bias of these parameters. Although a bias removal of the Stokes vector was suggested for DOPU, none has been suggested for other parameters. Here, we present a bias correction of von Neumann entropy. We derive equations to estimate the entropy of the random noise. It is subtracted from the measured entropy to calculate the entropy that includes only the polarization property of the sample. The theory is validated by measurements of a glass plate and waveplates, and is applied to the imaging of a healthy human eye anterior segment as an image filter.

10053-103, Session PMon

Tissue dispersion measurement techniques using optical coherence tomography

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Dispersion, a result of wavelength-dependent index of refraction variations, causes pulse-width broadening with detrimental effects in many pulsed-laser applications. It is also considered to be one of the major causes of resolution degradation in Optical Coherence Tomography (OCT). However, dispersion is material dependent and, in tissue, Group Velocity Dispersion (GVD) could be used, for example, to detect changes associated with early cancer and result in more accurate disease diagnosis. In this summary we compare different techniques for estimating the GVD from OCT images, in order to evaluate their accuracy and applicability in highly scattering samples such as muscle and adipose tissue. The methods investigated included estimation of the GVD from (i) the point spread function (PSF) degradation, (ii) the shift (walk-off) between images taken at different center wavelengths and (iii) the second derivative of the spectral phase. The measurements were degraded by the presence of strong Mie scattering and speckle noise with the most robust being the PSF degradation (standard deviation of 10-25 %) and the least robust the phase derivative method (inapplicable to highly scattering tissues). If the GVD is to be used to provide sensitive diagnostic information from highly scattering human tissues, it would be preferable to use the resolution degradation as an estimator of the GVD.

10053-104, Session PMon

Speckle reduction of OCT images using an adaptive cluster-based filtering

Saba Adabi, Elaheh Rashedi, Qiuyun Xu, Ali Hariri, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States)

ptical coherence tomography (OCT) has become a favorable device in the dermatology discipline due to its high resolution and moderate penetration depth. OCT images however contain grainy noise like pattern, called speckle, attributable to the broadband source that has been used in the configuration of OCT. So far, a variety of software based techniques is introduced to reduce speckle in OCT images. Most of these methods are generic and can be applied to OCT images of different tissues. Considering the architectural structure of skin layers, the skin image can benefit from being segmented in to differentiable clusters, and being filtered separately in each cluster using a filtering methods such as Wiener. We successfully developed a cluster based adaptive wiener filter (CWF) which can enhance the OCT images employing fundamental characteristic of OCT images; their optical properties as well as their intensity statistical information.

The algorithm starts with a hierarchical agglomerative clustering method and subsequently, groups the pixels of the image into different clusters. An adaptive wiener filter is devised on clusters based on calculating the mean, the variance and the noise variance of each cluster separately. The proposed algorithm is tested on several fabricated optical solid phantom with predetermined optical properties as well as healthy skin images. The quantitative analysis show that the proposed cluster-based filtering method increase the signal-to-noise ratio and contrast to noise ratio while preserving the edges in the image

10053-105, Session PMon

Segmentation of OCT retinal images by a convolution neural network

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Segmentation of retinal images obtained by optical coherence tomography has been performed by many techniques, including Canny edge detection, graph cut, and other segmentation methods. Commercial OCT retinal scanners generally have segmentation capability. Missing from this list of retinal segmentation techniques are neural network methods. Neural network computation has become a very promising and commercially active area in the past few years. Google's DeepMind organization is teaming up with Moorfields Eye Hospital to analyze 1M OCT retinal images by deep learning methods. We present a simple convolutional neural network for segmenting OCT retinal images. It was implemented using Google's TensorFlow programming framework. The purpose of this poster is to put this technique in context with other methods and to investigate whether neural network methods might have a place in this field.

10053-106, Session PMon

Dual-beam angular compounding for speckle reduction in optical coherence tomography

Wei Cheng, Jie Qian, Xinjian Chen, Jianhua Mo, Zhaoyuan Cao, Li Li, Soochow Univ. (China)

Optical coherence tomography (OCT) as a novel optical imaging technique has been undergoing a rapid development in the past decades, which utilizes coherence properties of optical waves backscattered from samples. It can produce cross-sectional images with both axial and lateral resolutions at micrometers. Due to the coherent properties of the image formation process, speckle noise is inevitably present in OCT images. Speckle noise can obscure small scattering structures and limit the effective spatial resolution. In this paper, we propose a novel dual-beam angular compounding method to reduce speckle noise of OCT image without losing imaging speed and spatial resolution. Two parallel light beams are focused into sample at different angles. The epi-detection scheme creates three different combinations of the two light beams above, which produce three images in single B-scan. The three images are separated in depth, allowing for averaging the three images to reduce speckle noise. The results show that our dual-beam method can achieve a 1.56-fold improvement in speckle contrast, which is comparable to that (1.6-fold) by 16 adjacent B-scans averaging. Further improvement (2.42-fold) was achieved by combining our method with five adjacent B-scans averaging. The results above demonstrated that our method is an effective way to reduce speckle noise in OCT image.

10053-107, Session PMon

Optical coherence tomography with pre-calculated reference spectra

Yi Wang, Xiaodong Chen, Haitao Liang, Daoyin Yu, Tianjin Univ. (China)

Optical coherence tomography (OCT) is a non-invasive and high-resolution technology for cross-sectional imaging based on the principles of low-coherence interferometer. Spectral-domain OCT (SD-OCT) uses spectrometer or swept source to acquire interference spectrum of sample and reference light. The uniformity of spectra in wavenumber space is critical for high-precision imaging. And in spectral-domain OCT data processing, resample algorithms are widely used to achieve the uniformity. But these resample algorithms are often sophisticated, and their precision is dependent on the method and equipment. In this paper, we proposed an OCT without inverse FFT. A series of reference spectra corresponding to different optical path length difference was used to convolve with spectra gotten by OCT to acquire time-domain tomography instead of inverse FFT, thus eliminating the resample of spectra. The reference spectra were calculated according to the laser source parameters before imaging and corrected with correction spectrum from sample arm to compensate the influence of sample arm. Experiment was done with a mirror as sample and validated our setup.

10053-108, Session PMon

Volumetric vessel reconstruction method for absolute blood flow velocity measurement in Doppler OCT images

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Doppler optical coherence tomography (DOCT) is considered one of the most promising functional imaging modalities for neuro biology research and has demonstrated the ability to quantify cerebral blood flow velocity at a high accuracy. However, the measurement of total absolute blood flow velocity (BFV) of major cerebral arteries is still a difficult problem since it not only relates to the properties of the laser and the scattering particles, but also relates to the geometry of both directions of the laser beam and the flow. In this paper, focusing on the analysis of cerebral hemodynamics, we presents a method to quantify the total absolute blood flow velocity in middle cerebral artery (MCA) based on volumetric vessel reconstruction from pure DOCT images. A modified region growing segmentation method is first used to localize the MCA on successive DOCT B-scan images. Vessel skeletonization, followed by an averaging gradient angle calculation method, is then carried out to obtain Doppler angles along the entire MCA. Once the Doppler angles are determined, the absolute blood flow velocity of each position on the MCA is easily found. Given a seed point position on the MCA, our approach could achieve automatic quantification of the fully distributed absolute BFV. Based on experiments conducted using a swept-source optical coherence tomography system, our approach could achieve automatic quantification of the fully distributed absolute BFV across different vessel branches in the rodent brain.

10053-109, Session PMon

Coagulation monitoring based on blood elastic measurement using optical coherence tomography

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Blood coagulation is a process during which blood forms a clot from a liquid. Disorders of coagulation can result in life-threatening bleeding or obstructive clotting. The assessment of coagulation function is significant

for diagnosis of thrombophilia and hemophilia, risk prediction of bleeding and prevention of thrombotic disorder. In this study, we developed an optical coherence elastography (OCE) system to dynamically monitor blood coagulation and quantitatively determine the coagulation function by blood elastic measurement. When blood forms a clot from a liquid, ultrasonic force induces a shear wave, which is detected by optical coherence tomography (OCT). The coagulation of porcine whole blood recalcified by calcium chloride is assessed using the metrics of reaction time, clot formation kinetics and maximum shear modulus. The OCE system can noninvasively monitor the blood coagulation and quantitatively determine the coagulation function.

10053-110, Session PMon

Spectroscopic optical coherence tomography for absorption quantification in the blue wavelength region

Colin Veenstra, Lot Jeurink, Ivo M. Vellekoop, Nienke Bosschaart, Univ. Twente (Netherlands)

The optical absorption of blood in the visible wavelength range can be used to estimate clinically important chromophore concentrations, such as bilirubin (absorption peak around 460nm) and hemoglobin. Increased bilirubin levels induce jaundice and decreased hemoglobin levels may lead to anemia. To accurately quantify the optical absorption in blood, localized measurements are required due to the inhomogeneous distribution of blood vessels.

Spectroscopic optical coherence tomography (sOCT) has proven its potential for localized and quantitative measurements of the spectrally resolved optical absorption in tissue. However, due to limited availability of single mode, broadband light sources in the visible region and stronger system sensitivity roll-off for shorter wavelengths, it remains a challenge to quantitatively measure optical absorption around the short edge of the visible region (i.e. in the blue) using sOCT.

We developed a Fourier domain sOCT system for quantitative measurements of optical absorption in the wavelength range of 440nm-650nm. Frequency modulation of the reference arm field allows measurements around zero-delay at any depth inside the sample. Due to finite spectrograph pixel size, lower frequent fringes —i.e. smaller path length difference between both arms — are sampled more accurately. As a consequence, measurements at zero-delay yield higher system sensitivity.

We validated our system on phantoms consisting of Ecoline ink and Intralipid with absorption values resembling human whole blood. In measurements on whole blood, we demonstrated the capability of our system to quantify chromophore concentrations with an accuracy of within 10%.

10053-111, Session PMon

Collagen birefringence assessment in heart chordae tendineae through PS-OCT

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Degenerative mitral regurgitation is a serious and frequent human heart valve disease. Malfunctioning of this valve brings the left-sided heart through a significant increase of pressure and volume overload. Severe degenerative mitral incompetence generally requires surgical repair or valve replacement with a bioprosthesis or mechanical heart valve.

Degenerative disease affects the leaflets or/and the chordae tendineae, which link the both leaflets to the papillary muscles. During mitral valve surgical repair the valve leaflets, annulus and chordae are reconstructed in order to prevent postoperative recurrence of valve regurgitation. The operative evaluation of the diseased and apparently normal chordae tendineae mainly depends of the surgeon´s experience, without any other objective diagnosis tool.

In this work, PS-OCT is applied for the first time to evaluate the condition of human chordae coming from the mitral valve. It consists on a prospective study to test the viability of this technique for the evaluation of the collagen core of chords. This core presents a strong birefringence due to the longitudinal and organized arrangement of its collagen bundles Collagen core with different density provides different birefringence indicators and measurements, being an objective marker of the core structure.

Ex-vivo mitral degenerative chordae tendineae has been analyzed with PS-OCT. Intensity OCT is used to obtain complementary morphological information of the chords. Birefringence results correlate with the previously reported values for human tendinous tissue.

10053-112, Session PMon

Depth-encoded dual beam Phase-Resolved Doppler OCT for Doppler-angle-independent flow velocity measurement

Jie Qian, Wei Cheng, Zhaoyuan Cao, Xinjian Chen, Jianhua Mo, Soochow Univ. (China)

Phase-resolved Doppler optical coherence tomography (PR-D-OCT) is a functional OCT imaging technique that can provide fast and high-resolution depth-resolved measurement on flow in biological materials. However, a common problem with conventional PR-D-OCT is that this technique often measures the flow motion projected onto the OCT beam path. Without knowing the projection angle ? between vessel orientation and the OCT beam path, it is hard to obtain the absolute flow velocity. Moreover, the projection angle is usually missing for in vivo measurement on human blood vessel, which obscures quantitative study on human blood flow. In this paper, we proposed a novel dual-beam PR-D-OCT method to measure absolute flow velocity, which does not require knowing the projection angle. The sampling beam is divided into two equal beams and separated laterally before being focused into samples, which leads to two different incident angles. The images by the two beams are encoded to different depths in individual B-scan. Thus, upon single B-scan, with the Doppler signals measured by the two beams and the difference between the incident angles of the two beams, we can calculate the absolute flow velocity. We validated our approach in vitro on an artificial flow phantom on our home-built 1060 nm swept source OCT. The flow phantom is a capillary with 300-?m diameter perfused with milk at constant speed by a syringe pump. Experimental results demonstrated that our method can provide an accurate measurement of absolute flow velocity without the projection angle.

10053-113, Session PMon

Gold nanoparticles evaluation using functional optical coherence tomography

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The main object of this research was to assess the ability to characterize the gold nanoparticles using optical modalities like optical coherence tomography. Since the nanoparticles, especially gold one, have been very attractive for medical diagnosis and treatment the amount of research activities have been growing rapidly. The nanoparticles designed for different applications like contrast agents or drugs delivery change the optical features of tissue in different way. Therefore, the expanded analysis

of scattering optical signal may lead to obtain much more useful information about the tissues health and the treatment efficiency. The noninvasive measurements of the concentration and distribution of the nanoparticles, as well as their size in the media have been taken under consideration. For this purpose the polarization sensitive optical coherence tomography system with spectroscopic analysis (PS-SOCT) has been designed and used. In this contribution we are going to present the PS-SOCT measurement data obtained for the gold nanoparticles. The measurements have been taken for the solid (PDMS matrix) and liquid (gold nanoparticles in water) samples having different particles concentrations and sizes. The case of making nanoparticles agglomerations has also been studied.

10053-114, Session PMon

improvement of velocity dynamic range for slow motion detection without reduction of line period using optical Doppler tomography

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The objective of this study is to improve the velocity dynamic range (VDR) for the slow motion detection in spectral domain optical doppler tomography (SD-ODT) quantitatively without reduction of line period while maintaining the maximum detector speed. For motion detection in A-scan imaging, a linear step motor is used for sample movement. Also the syringe pump connected to a syringe and a micro tube are used for B-scan imaging, to evaluate the performance of multiple A-lines. The mobility of samples is detected from the phase difference between two A-lines, using the basic principles of Doppler frequency shift and Kasai's autocorrelation algorithm. The proposed method enables to change the line interval of two compared A-lines. In the proposed method, the comparison between two a-lines can be taken far apart from each other, which can be chosen accordingly to match the flow velocity. This enables a dynamic range of possibilities to obtain phase difference between two a-lines, thus enabling the system to detect slow moving samples. We built ultrafast signal processing software for real-time imaging using both general-purpose graphics processing unit (GP-GPU) with CUDA programming and CPU's multi-threading technique. The results are expressed to color Doppler imaging and numerical value. Hence, our SD-ODT system can acquire slow motion in moving samples without performance degradation. Also, It is suitable for flow measurements in blood vessel or the capillaries, where both high resolution imaging and the detection of slow motion is essential.

10053-115, Session PMon

Rat brain imaging using full field optical coherence microscopy with short multimode fiber probe

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We demonstrated FF OCM (full field optical coherence microscopy) using an ultrathin forward-imaging SMMF (short multimode fiber) probe of 50 ?m core diameter, 125 ?m diameter, and 7.4 mm length, which is a typical graded-index multimode fiber for optical communications. The axial resolution was measured to be 2.20 ?m, which is close to the calculated axial resolution of 2.06 ?m. The lateral resolution was evaluated to be 4.38 ?m using a test pattern. Assuming that the FWHM of the contrast is the DOF (depth of focus), the DOF of the signal is obtained at 36 ?m and that of the OCM is 66 ?m. The contrast of the OCT images was 6.1 times higher than that of the signal images due to the coherence gate. After an euthanasia the rat brain was resected and cut at 2.6mm tail from Bregma. Contacting SMMF to the primary somatosensory cortex1 and the agranular insular cortex2 of ex vivo brain, OCM images of the brain were measured 100 times with 2?m step. In the depth image of 3D images (38?m-diameter, 150?m-depth) in cortex1 the layer structure with the periods of 8-11?m could be seen. In the depth image in cortex2 the tilted layer structure with the longer periods of 22-28?m could be also seen. All measurements have been done within 2.5 hours after the resection. Further investigations in details are needed. The feasibility of an SMMF has been demonstrated.

10053-116, Session PMon

Phase-stabilized swept-source OCT for the study of neurovascular coupling

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Recent development of optical coherence tomography (OCT) angiography enables depth-resolved 3D imaging of cerebral microvascular network in living animals without using an extrinsic contrast agent. Nevertheless, functional details of cerebral microvasculature has been rarely reported since the volumetric imaging rate of conventional OCT is not sufficient for capturing fast cerebral blood flow (CBF) changes, which occur in a few seconds. Currently, no standard method exists for quantification of CBF using OCT because of technical challenges. While several methods have been suggested to measure blood flow quantitatively in the rodent brain using Doppler OCT or intensity variance analysis, there are few studies that utilize the capability of high speed swept source OCT for investigation of neurovascular coupling. In this research, we develop a high speed phase-stabilized swept source OCT system to visualize CBF changes with high spatial (~ 10 um) and temporal resolution (~1 s). A fiber ring cavity-based wavelength-swept laser with a center wavelength of 1.3 um and an A-scan rate of 240 kHz was used for this study. By utilizing this system, we perform quantitative measurement of CBF changes at the microscopic level during neuronal activation in the rat somatosensory cortex.

10053-117, Session PMon

Characterization of reflectance-mode iNIRS system parameters and in vivo comparison with CW-NIRS

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Continuous wave near-infrared spectroscopy (CW-NIRS) aims to probe the physiology of highly scattering biological tissues non-invasively with near-infrared light by measuring changes in the absorption coefficient of tissues at various wavelengths to obtain oxy- and deoxy- hemoglobin concentration changes dynamically. Current challenges in the field of NIRS are the large number of assumptions to quantify baseline flow and oxygenation from continuous wave (CW) measurements, the cost of more robust time-domain (TD-NIRS) and frequency-domain (FD-NIRS) methods that enable baseline measurements, and the current requirement for complex multimodality instrumentation to measure oxygen metabolism. Interferometric near-infrared spectroscopy (iNIRS) is a time-of-flight resolved sensing method that was recently introduced for determination of optical and dynamic properties of a turbid medium. iNIRS measures the interference spectrum of light traversing the turbid medium using a rapidly tunable, or frequency swept, light source. The performance of iNIRS critically depends on the source and detection apparatus. Here, we experimentally characterize

iNIRS system parameters, including sensitivity, dynamic range, bandwidth (tuning range), dynamic coherence time, and instrument response function (IRF), using a current-tuned 855 nm distributed feedback (DFB) laser as the light source. We introduce a novel Mach-Zehnder interferometer variant with a multi-pass loop to efficiently measure the dynamic coherence time of the rapidly tuned laser at a tuning speed of 50 kHz. Finally, the brain of a nude mouse under hypercapnia is continuously monitored, and absolute absorption measurements obtained from iNIRS are used to derive absorption changes, which are then validated against absorption changes determined by CW-NIRS.

10053-118, Session PMon

Demonstration of the usefulness of optical coherence tomography in imaging a mouse tail model of lymphedema

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To investigate the usefulness of optical coherence tomography (OCT) for imaging lymphedema, we directly compared it to other histological methods in a mouse model of lymphedema. We performed detailed imaging of the lymphedema lesion on a mouse tail. We collected samples of the mouse tail for OCT and created histopathological samples. We constructed a spectrometer-based OCT system using a fiber-optic Michelson interferometer. The light was directed to 50:50 couplers that split the light into reference and sample arms. Backscattered light from a reference mirror and the sample produced an interference fringe. An OCT image of the lymphedema model revealed an inflammatory reaction of the skin that was accompanied by edema, leading to a reduction in the light attenuation coefficient in the subcutaneous layer. Correspondingly, histological biopsy showed an inflammatory response that involved edema, increased neutrophils in epidermis and subdermis, and lymphatic microvascular dilatation. Furthermore, the lymphedema model showed an increase in thickness of the dermis in both diagnostic studies. In the mouse tail model of lymphedema, OCT imaging showed reciprocative results to other histological examinations. OCT provides a quick and useful diagnostic imaging technique for lymphedema and is a valuable addition or complement to other noninvasive imaging tools.

10053-120, Session PMon

A novel dermo-epidermal localization algorithm for swept source OCT images of human skin

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Optical coherence tomography (OCT) is a noninvasive diagnostic method that offers a view into the superficial layers of the skin in vivo in real-time. OCT delivers morphological images of microstructures within the skin. Epidermal thickness is OCT images is of paramount importance, since dermo-epidermal junction (DEJ) location alteration is the start of several skin abnormalities. Due to the presence of speckle noise, devising an algorithm for locating DEJ in the OCT images is challenging. In this study we propose a semi-automatic DEJ detection algorithm based on graph theory that is resistant to speckle. In this novel approach we use attenuation map as a complementary feature compared to the previous methods that are mainly based on the intensity information. The method is based on converting border segmentation problem to the shortest path problem using graph theory. To smooth boarders, we introduced a thinning fuzzy system enabling closer match to manual segmentation. Subsequently, an averaged A-scan analysis is performed to obtain the mean epidermal thickness. The DEJ detection method is performed on 96 B-Scan OCT skin images taken from different sites of body of healthy individuals. The results are evaluated based on several expert's visual analysis.

10053-121, Session PMon

Classification of human ovarian tissue using full field optical coherence tomography

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In this study, the feasibility of a full-field optical coherence tomography (FFOCT) system for rapid wide field optical analysis of normal and malignant human ovarian tissue pathologies was demonstrated. Fixed ovarian tissue samples were imaged ex vivo with the FFOCT system and compared with the corresponding H&E stained histology slides. A total of 55 images from 14 ovarian tissue samples (7 normal, 7 malignant) were analyzed and five features such as mean, variance, skewness, kurtosis and entropy were extracted based on the image histogram. Using a generalized linear model (GLM) for classification, a sensitivity of 91% and specificity of 86% were achieved. This indicates that the FFOCT system can be a very useful tool for rapid, label free optical pathology of ovarian tissue and can reduce the time and corresponding cost associated with diagnosis of ovarian cancer.

10053-122, Session PMon

Utilizing optical coherence tomography to assess oral cancer in a low resource settings

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Oral cancer is the sixth most common cancer worldwide; more than 2/3 of cases are identified at a late stage, when prognosis and treatment outcomes are poor with less than 15% survival over 5 years. Oral cancer is currently detected by visual inspection of oral tissue under visible light with further diagnosis of punch biopsy and histopathological analysis. Such a method for diagnosing benign, pre-malignant, and malignant oral lesions is invasive, resource intensive and poorly suited to monitoring suspect lesions. We have shown that Optical Coherence Tomography (OCT), a noninvasive, non-ionizing tomographic imaging technique can indeed differentiate tissue malignancy. By analyzing the OCT intensity spatial frequency fluctuations in OCT images we are able to classify cancer state. We have created a mobile imaging system and 1D scanning probe that we have deployed in Bangalore, India where oral cancer prevalence is among the highest worldwide. We have collected and analyzed 38 subject's OCT data with

corresponding histopathology to assess our image processing algorithm detection accuracy. We have shown through our initial human clinical trial that the sensitivity and specificity of our OCT imaging-processing algorithm to differentiate between oral cancer and healthy tissue is 85% and 90% respectively.

10053-123, Session PMon

Textural analysis of optical coherence tomography skin images: quantitative differentiation between healthy and cancerous tissues

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Optical Coherence Tomography (OCT) offers real time high resolution three dimensional images of tissue microstructures. Texture in skin varies in different parts of the body according to specific functional need. In this study, we used OCT skin images acquired from ten volunteers, neither of whom had any skin conditions addressing their anatomic location. OCT segmented images are analyzed based on their optical properties (attenuation rate) and 62 image statistical textural features e.g., contrast, correlation, homogeneity, energy, entropy. Utilizing the information and referring to their clinical insight, we aim to make a computational model for the healthy skin. The derived parameters represent the OCT microstructural morphology characterization and might provide biological information for generating an atlas of normal skin from different anatomic sites of human skin and may allow for the cell microstructural changes in cancer patients and help in the adjustment of treatment based on the normal model. We then compared the parameters of healthy samples with those of abnormal skin and classified them with 80 % sensitivity.

10053-124, Session PMon

An efficient method for classifying skin tumors based on the two-dimensional Fourier fractal analysis

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Optical coherence tomography (OCT) is currently employed in the diagnosis of the skin tumors. Generally speaking, quantitative image features extracted from OCT images have already been used as indicators to classify the skin tumors. Particularly, the fractal dimension could provide an efficient way in the analysis of OCT images of the skin tumors. In the present paper, the two dimensional Fourier fractal analysis was performed on OCT images for automatically classifying the melanomas, basal cell carcinomas and pigment nevi. Generalized estimating equations were used to test for differences between the skin tumors. A modified p value of <0.001 was considered statistically significant. Significant decrease of the fractal dimension was observed in basal cell carcinomas and pigment nevi as compared with melanomas. Our results suggested that the two-dimensional Fourier fractal analysis could provide a more efficient method to differentiate basal cell carcinomas and pigment nevi from melanomas as compared to the two dimensional differential box-counting method. Further research is warranted to determine how this approach may be used to improve the classification of the skin tumors.

10053-125, Session PMon

Functional optical coherence tomography for hard tissue evaluation

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The main object of this research was to assess the ability to use the polarization sensitive optical coherence tomography with spectroscopic analysis (PS-SOCT) for hard tissue evaluation. The hard tissue resorption and degradation process is extremely important for medical treatment, especially for regenerative medicine. The high complexity of the hard tissue monitoring and the lack of methods, which can be used on demand or constantly without any harm to the patient, makes this issues more interesting to investigate. In this contribution the Authors have presented the new approach in hard tissue diagnosis based on functional optical coherence tomography. The PS-SOCT signal delivers lot of valuable information about evaluated tissue, which are related to not only the intensity of the backscattered light gathered from particular points inside the scanning volume but also hidden in its spectral characteristic and state of polarization. The correlation between tissue degradation and PS-SOCT recorded data has been studied by the use of human tooth and small mammals bones. The tissues have been put under influence of phosphoric (V) acid and the degradation process has been monitored by PS-SOCT system. Obtained data have shown the correlation between the degree of degradation and demineralization of the tissues and their optical properties measured by PS-SOCT.

10053-127, Session PMon

Characterization of the drying progression of sessile droplets of a Sunset Yellow water solution using optical coherence tomography

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The drying process and final deposition patterns of sessile droplets of an aqueous lyotropic chromonic liquid crystal (LCLC) are investigated using optical coherence tomography. Real time cross-sectional data allow us to observe the effects of nonuniform evaporation, which creates concentration gradients that cause nematic and columnar liquid crystal phases to transit from the edge of the droplet to its center. Concentration gradients at the droplet-air interface also cause a reversed convective flows, knows as the "coffee-ring effect", which is made visible by the movement of polystyrene micro particles. Counteracting mechanisms against convective flows lead to a volcano-like deposition pattern, that resembles, but is structurally different from a "coffee-ring" pattern. These results, all evident in OCT images, confirm OCT as a valuable tool in assessing deposition phenomena and liquid dynamics of sessile droplets.

10053-128, Session PMon

3D printing based on cellular level optical coherence tomography images

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Three-dimension (3D) printing, or stereolithography, is a revolutionary technology providing rapid prototyping and manufacturing, which has a significant effect on education, industry and biomedicine. Although the biomedical application of 3D printing has been developed to produce stents, splints, and even bones, current technology mainly 3D print manually designed structures instead of real time or quasi-real time scanned images. It would be ideal to 3D print cellular level tissue structures according to the 3D images of the in vitro and in vivo samples. To obtain 3D cellular level tissue structure files to support the 3D printing, a suitable 3D imaging technique is especially critical. Existing computed tomography (CT) and magnetic resonance imaging (MRI) can provide 3D images of good penetration depth with large size structures, however their millimeter scale axial and lateral resolutions prevent their applications in cellular level tissue imaging, processing, and printing. Optical coherence tomography (OCT) is an advantageous non-invasive cross sectional imaging technology, providing much higher axial and lateral resolutions than CT and MRI for 3D viewing of tissue structures. We have recently improve OCT axial and lateral resolutions to about 3 μm by digital image processing technology, facilitating high quality cellular level 3D imaging to support 3D printing. The axial resolution improvement is accomplished by using a broadband superluminescent diode light source of 100 nm spectral bandwidth at 850 nm center wavelength and a compatible optical spectrometer followed by deconvolution processing. The lateral resolution improvement is achieved by multi-frame superresolution technique using a 60 mm focal length lens. A special high dynamic range (HDR) technique is also introduced to improve the OCT image brightness uniformity over the whole imaging depth range. The high quality 3D OCT image is then transformed to a 3D triangular mesh file, with millions of simple polygons to form the surface, for 3D printing. The effectiveness of our technique is verified by 3D printing of onion tissue structures showing clear cell structures. The OCT image enabled 3D printing should benefit future 3D bioprinting of tissues and organs.

10053-29, Session 5

Full depth human cutaneous vasculature imaging using multimodal OCT/OCTA/ photoacoustic tomography for diagnosis of skin disorders

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We present in this work a multi-modal optical imaging system combining optical coherence tomography (OCT), OCT angiography (OCTA) and photoacoustic tomography (PAT). This multi-modal system enables us to extract human cutaneous vasculature for up to 6 mm by fusing OCTA and PAT. Skin morphology is provided at the same time by OCT. The system features a scanning probe mounted on a portable rack, which permits access to nearly all parts of human body. Applying the system in skin imaging, we acquired for the first time to our knowledge the full blood vessel network for various skin disorders. The information of several diseases are shown in this paper, including nevus araneus on dorsum of hand, purpura on the thigh and naevus flammeus on the thigh. Comparing the vascular network in the pathological region to normal region, it is clear that our OCT/OCTA/PAT system has a significant value for non-invasive investigation of a variety of skin conditions.

10053-30, Session 5

Automated computational aberration correction method for OCT and OCM

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Aberrations in an optical system cause a reduction in imaging resolution and poor image contrast, and limit the imaging depth when imaging biological samples. Computational adaptive optics (CAO) provides an inexpensive and simpler alternative to the traditionally used hardware-based adaptive optics (HAO) techniques. In this paper, we present an automated computational aberration correction method for broadband interferometric imaging techniques, e.g. optical coherence tomography (OCT) and optical coherence microscopy (OCM). In the proposed method, the process of aberration correction is modeled as a filtering operation on the aberrant image using a phase filter in the Fourier domain. The phase filter is expressed as a linear combination of Zernike polynomials with unknown coefficients, which are estimated through an iterative optimization scheme based on maximizing an image sharpness metric. The Resilient backpropagation (Rprop) algorithm, which was originally proposed as an alternative to the gradient-descent-based backpropagation algorithm for training the weights in a multilayer feedforward neural network, is employed to optimize the Zernike polynomial coefficients because of its simplicity and the robust performance to the choice of various parameters. Stochastic selection of the number and type of Zernike modes is introduced at each optimization step to explore different trajectories to enable search for multiple optima in the multivariate search space. The method was validated on various tissue samples and shows robust performance for samples with different scattering properties, e.g. a phantom with subresolution particles, an ex vivo rabbit adipose tissue, and an in vivo photoreceptor layer of the human retina.

10053-31, Session 5

Optically subsampled optical coherence tomography (OCT)

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Current implementations of OCT can image over long depth ranges with slower imaging speeds, or at high imaging speeds with more limited depth ranges; simultaneous high-speed and long depth range imaging is restricted by limitations in the electronic bandwidth of current digitizers and data transfer buses. The inability of current OCT systems to provide megahertz-scale speeds over centimeter-scale depth ranges has hindered its utility in sites with complex geometries and expansive fields. Here we describe a new optically subsampled architecture for OCT that circumvents these technical barriers. The architecture includes multiple technological innovations including novel methods for echo-delay ranging, new algorithms for visualizing three-dimensional information at video-rates, and high-speed methods for optical quadrature demodulation. The presented architecture also includes a novel laser design based on intracavity dispersion-stretched pulses that allows operation in excess of 10 MHz with centimeter-scale coherence lengths and up to 100 nm bandwidth. Here, we describe the operating principles of this architecture and present examples of its use including a polarization-sensitive implementation that allows video-rate mapping of vectorial birefringence in intraoperative settings and wide-field

imaging of the oral cavity and ex vivo GI tract tissues. These results serve to demonstrate the broad potential of this optically subsampled imaging platform, highlighting near and long-term opportunities for the technology in both clinical and experimental settings.

10053-32, Session 5

Stimulated Raman scattering spectroscopic optical coherence tomography

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Optical coherence tomography (OCT) enables non-invasive, high-resolution, tomographic imaging of biological tissues by leveraging principles of low coherence interferometry; however, OCT lacks molecular specificity. Spectroscopic OCT (SOCT) overcomes this limitation by providing depth-resolved spectroscopic signatures of chromophores, but SOCT has been limited to a couple of endogenous molecules, namely hemoglobin and melanin. Stimulated Raman scattering, on the other hand, can provide highly specific molecular information of many endogenous species, but lacks the spatial and spectral multiplexing capabilities of SOCT. In this work we integrate the two methods, SRS and SOCT, to enable simultaneously multiplexed spatial and spectral imaging with sensitivity to many endogenous biochemical species that play an important role in biology and medicine. The method, termed SRS-SOCT, has the potential to achieve fast, volumetric, and highly sensitive label-free molecular imaging, which would be valuable for many applications. We demonstrate the approach by imaging excised human adipose tissue and detecting the lipids' Raman signatures in the high-wavenumber region. Details of this method along with validations and results will be presented.

10053-33, Session 5

Self-interference epi-fluorescence microscopy with three-phase detection for depth-resolved confocal fluorescence imaging

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A novel method for depth-resolved three-dimensional confocal fluorescence imaging is presented based on self-interference fluorescence microscopy (SIFM). In SIFM fluorescent light is collected in epi-detection and is sent through a phase plate, i.e. a glass plate with a hole, that transforms the fluorescent wavefront curvature into a phase of a spectrally resolved interference pattern. Analysis of the SIFM spectral phase therefore gives information on the depth position of a fluorescent sample. In this paper a method is described for interferometric three-phase detection of the SIFM signal using photon-counter detectors for improved detection sensitivity. A Mach-Zehnder interferometer (MZI) based on a 3?3 fiber-coupler was developed to impose a spectral modulation onto an SIFM signal in order to create three phase-shifted output signals for which the intensities alternately oscillate as a function the SIFM spectral phase. As such the MZI signal outputs encode directly for the amplitude and phase parameters of the SIFM signal and can be used for the axial localization of fluorophores. The response of the SIFM setup on the axial position of a sample was demonstrated with a layer of immobilized fluorescent microspheres that were moved through the focus with a motorized translation stage. This experiment showed the expected alternating detector behavior and a linear dependence of the SIFM phase with depth over an axial range of 410 μm for a 4? microscope objective. In addition a capillary blood vessel phantom constructed out of dye-soaked absorbent fibers was imaged to show the potential of SIFM for three-dimensional fluorescence angiography.

10053-34, Session 5

3D wide field-of-view Gabor-domain optical coherence microscopy advancing real-time in-vivo imaging and metrology

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Real-time volumetric high-definition wide-field-of-view in-vivo cellular imaging requires micron-scale resolution in 3D. Compactness of the handheld device and distortion-free images with cellular resolution are also critically required for on-site use in clinical applications. By integrating a custom liquid lens-based microscope and a dual-axis MEMS scanner in a compact handheld probe, Gabor-domain optical coherence microscopy (GD-OCM) breaks the cellular lateral resolution limit of optical coherence tomography, enabling advances in biotechnology. Distortion-free imaging with no post-processing is achieved with a compact, lightweight handheld MEMS scanner that obtained a 12-fold reduction in volume and 17-fold reduction in weight over a previous dual-mirror galvanometer-based scanner. Approaching the holy grail of medical imaging – noninvasive real-time imaging with histologic resolution – GD-OCM demonstrated invariant resolution of 2 μm throughout a volume of 1x1x0.6 mm^3, acquired and visualized in less than 2 minutes with fast processing on graphics processing units. Results on the metrology of manufactured materials and characterization of tissue with GD-OCM are presented.

10053-35, Session 6

Multi-channel joint aperture Doppler OCT for investigation of ocular blood flow

Andreas Wartak, Richard Haindl, Florian Beer, Bernhard Baumann, Michael Pircher, Christoph K. Hitzenberger, Medizinische Univ. Wien (Austria)

A new approach for the quantification of human ocular blood flow (OBF) by the means of multi-channel delay encoded swept source joint aperture Doppler optical coherence tomography (SS-JA-D-OCT) is introduced. We developed an experimental setup based on the principles of three-beam OCT, joint aperture OCT and path length encoding, enabling three-dimensional (3D) velocity vector reconstruction of moving particles without prior knowledge on the direction of motion. In contrast to previous multi-channel SD-D-OCT approaches, we are applying only one active illumination beam onto the sample while simultaneously probing the backscattered light from two further, linear independent, angular orientations. Thus, we are no longer obliged to divide illumination power among three beams to satisfy corresponding laser safety requirements. We primarily demonstrate our system's performance via in vitro flow phantom measurements utilizing a glass capillary perfused with a scattering fluid. Furthermore, in vivo validation measurements at retinal vessel bifurcations in the eyes of healthy human volunteers were conducted. The final aim of this work is to investigate retinal perfusion quantitatively for diagnostic and monitoring purposes of ocular disease development and progression. Total retinal blood flow (TRBF) is associated to be a significant biomarker for ocular diseases linked to alterations in OBF like glaucoma, diabetic retinopathy (DR) or central/branch retinal vein occlusion (C/BRVO). Utilizing a circumpapillary scanning pattern around the optic nerve head (ONH), we were able to determine TRBF in the eyes of healthy human volunteers. The ultimate goal, to investigate eyes of patients suffering from referred pathologies remains future work.

10053-36, Session 6

Characterization of flowing blood cells using a novel OCT technique: rigorous three-dimensional computational study.

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We have developed a highly realistic, Maxwell-based, model of an existing experimental optical coherence tomography based approach for characterizing blood cells flowing through a microfluidic channel. The characterization technique is indirect as it relies upon the perturbation, by blood cells, of light back-scattered by specially designed highly scattering substrate. This is in contrast with characterization techniques which directly sense light back-scattered by the cells. Up until now, our hypothesis for distinguishing between different blood cell types has been based upon experimental measurements and knowledge of cell morphology.

The absence of a mathematical model capable of modelling image formation, when the wave nature of light is integral, has impeded our ability to validate and optimize the characterization method. Recently, such a model has been developed and we have adapted it to simulate our experimental system and blood cells. The model has the following features: the field back scattered by the sample, for broadband and arbitrary profile beams, is calculated according to Maxwell's equations; the sample is a deterministic refractive index distribution; the scattered and reference electric fields are explicitly interfered; single and multiple scattering are implicitly modeled; most system parameters of practical significance (e.g. numerical aperture or wavefront aberration) are included the model.

This model has been highly successful in replicating and allowing for interpretation of experimental results. We will present the key elements of the three-dimensional computational model, based upon Maxwell's equations, as well as the key findings of the computational study. We shall also provide comparison with experimental results.

10053-37, Session 6

Quantitative angle-independent flow measurement using relative standard deviation OCT

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Incorporating different data processing methods, Optical coherence tomography (OCT) has the ability for high-resolution micro-angiography and quantitative flow velocity measurement. However, OCT micro-angiography cannot provide quantitative measurement of flow velocity, and the velocity measurement based on Doppler OCT requires the determination of Doppler angles, which are difficult for whole vascular network. In this study, we report a relative standard deviation OCT (RSD-OCT) for the mapping of the flow velocity in a vascular network without the calculation of Doppler angle. From the theoretical analysis and experimental validation, the RSD-OCT is angle-independent and can quantify the flow velocity conveniently after a calibration.

10053-38, Session 6

Velocity-gradient effects in intensity-based dynamic light scattering optical coherence tomography

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Dynamic light scattering optical coherence tomography (DLS-OCT) provides spatially-resolved quantitative characterization of diffusive motion as well as the flow velocity field in systems that exhibit mass transport, such as blood flow in biological systems, by calculating the autocorrelation function of the complex OCT signal. We have developed intensity-based DLS-OCT (iDLS-OCT), which extends DLS methods to a broader range of wavelength-swept OCT systems that lack phase stability. It has been commonly believed that the use of the complex signal allows DLS-OCT to uniquely determine the diffusion, the axial, and the transverse components of the motion of the scatterers. In this work we show, however, that gradients in the axial velocity of scatterers exert a fundamental influence on the autocorrelation function even in well-behaved, non-turbulent flow. We derive the explicit functional relation between axial-velocity gradients and the autocorrelation function, and we show that the effects of the velocity field and its derivatives are intimately related and their contributions cannot be separated. Therefore, a single DLS measurement cannot univocally determine the velocity field. In the case of iDLS-OCT, determining the flow speed from the autocorrelation function of the intensity is also ill-defined, more so than in DLS-OCT. We finally show that when flow is mostly perpendicular to the interrogating beam, it is possible to disentangle the gradient contributions and find the true flow speed of the scatterers in a single iDLS-OCT measurement. This has broad applications, including quantitative angiography in the retina and the choroid, where the vessels of interest are mostly orthogonal to the beam.

10053-39, Session 6

Angular compounded optical coherence tomography angiography for flow contrast enhancement

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Optical coherence tomography angiography (OCTA) is a promising imaging modality that enables a label-free, high-resolution and high-contrast image of biological tissue microvasculature. Typically, the blood flow contrast is implemented by mathematically analyzing the temporal dynamics of light scattering, and setting a threshold to distinguish the dynamic blood flow from the static tissue bed. However, high flow contrast is degraded by the residual overlap that results in misclassification errors between dynamic and static signals. Our study has demonstrated that flow contrast can be enhanced using a single-shot angular compounded OCTA (AC-OCTA). Because a continuous modulation is induced by the offset that the probing beam is away from the beam center in the typical OCT sample arm, different incidence angles in the probing beam are encoded in B-scan modulation frequencies. The complex-valued spectral interferogram is reconstructed by removing the conjugate terms in the depth space and its Fourier transform along the transversal fast-scan direction generates a wide conjugate-free B-scan modulation spectrum in the full space of the spatial domain. By splitting the modulation spectrum, angle-resolved independent sub-angiograms are generated and then compounded to enhance the flow contrast. Both flow phantom and in vivo animal cerebral vascular imaging demonstrated that the proposed angular compounded OCTA can offer a ~50% decrease of misclassification errors and an improved flow contrast and vessel connectivity. This AC-OCTA is beneficial to facilitating the interpretation of OCT angiograms in clinical applications.

10053-40, Session 6

Real time OCT-based angiography device with hand-held probe

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This work is dedicated to development of the OCT system with angiography for everyday clinical use. Two major problems were solved during the development: compensation of specific tissue displacements, induced by contact scanning mode and physiological motion of patients (e.g. respiratory and cardiac motions) and on-line visualization of vessel net, to provide the feedback for system operator. The performance of the resulting OCT-based microangiography device with hand-held probe was evaluated by visualization of vessels nets of volunteers oral mucosa and skin on different locations (hands, face, abdomen etc.). Success-rate more than 90% was demonstrated during the experiments.

10053-41, Session 7

A theoretical model for optical oximetry at the capillary-level by optical coherence tomography

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Oxygen saturation (sO2) of RBCs in capillaries can indirectly assess local tissue oxygenation and metabolic function. For example, the altered retinal oxygenation in diabetic retinopathy and local hypoxia during tumor development in cancer are reflected by abnormal sO2 of local capillary networks. However, it is far from clear whether accurate label-free optical oximetry (i.e. measuring hemoglobin sO2) is feasible from dispersed red blood cells (RBCs) at the single-capillary level. The sO2-dependent hemoglobin absorption contrast present in optical scattering signal is complicated by geometry-dependent scattering from RBCs. Here we provide a theoretical model to calculate the backscattering spectra of single RBCs based on the first-order Born approximation, considering the orientation, size variation, and deformation of RBCs. We show that the oscillatory spectral behavior of RBC geometries is smoothed by variations in cell size and orientation, resulting in clear sO2-dependent spectral contrast. In addition, this spectral contrast persists with different deformations of RBCs, allowing the sO2 of individual RBCs in capillaries to be characterized. The theoretical model is verified by Mie theory and experiments using visible light optical coherence tomography (vis-OCT). Thus, this study shows for the first time the feasibility of, and provides a theoretical model for, label-free optical oximetry at the single-capillary level by backscattering-based imaging modalities, challenging the popular view that such measurements are impossible at the single-capillary level. This is promising for in vivo backscattering-based optical oximetry at the single-capillary level, to measure local capillary sO2 for early diagnosis, progression monitoring, and treatment evaluation of diabetic retinopathy and cancer.

10053-42, Session 7

Birefringence and vascular imaging of in vivo human skin by Jones-matrix optical coherence tomography

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Optical coherence tomography (OCT) is a real-time, noninvasive and high depth resolution imaging technique. These characteristics have enabled its use in a broad range of medical fields, such as ophthalmology, cardiology, gastroenterology, dentistry and dermatology. It is particularly suited to dermatology as skin is an easily accessible tissue of the human body.

Several types of functional OCT have been applied to dermatological investigation. Among them, polarization sensitive OCT (PS-OCT) and OCT angiography (OCT-A) are two of the most promising options. Currently, most of the dermatological PS-OCTs measure the phase retardation of skin. However, it was recently shown that the phase retardation measurement cannot be accurate for tissues with non-uniform birefringence axis (optic axis) orientation, such as skin. So, depth-localized birefringence measurement is required for dermatological investigation.

In this study, we demonstrate our new multifunctional (multi-contrast) Jones-matrix optical coherence tomography (JM-OCT). It is based on a similar design to our previous posterior-eye JM-OCT, but it uses a probe wavelength of 1310 nm, and the entire system is especially designed for in vivo skin imaging. The JM-OCT simultaneously measures the depth-resolved local birefringence tomography, degree-of-polarization-uniformity (DOPU), complex-correlation based OCT-A, as well as the scattering OCT. With these contrasts from entire optical properties, not only morphological structures, but also histological and functional characteristics such as collagen concentration and flow distribution are visualized.

10053-43, Session 7

Depth analysis of collagen directionality on axial human uterine cervical tissue using optical coherence tomography

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Uterine cervical collagen fiber network is vital to the normal cervical function in pregnancy. Previously, we presented an orientation estimation method to enable dispersion analysis on a single axial slice of human cervical tissue obtained from the upper half of cervix using optical coherence tomography (OCT). How the collagen fiber network structure changes from the internal os (top of the cervix which meets the uterus) to external os (bottom of cervix which extends into the vagina), remains unknown due to depth penetration limitations of OCT.

To establish a collagen fiber directionality “map” of the entire cervix, we imaged serial axial slices of human NP (n=11) and PG (n=2) cervical tissue obtained from the internal to external os using Institutional Review Board approved protocols at Columbia University Medical Center. Each slice was divided into four quadrants. In each quadrant, we stitched multiple overlapped OCT volumes and analyzed the en face images that were parallel to the surface. A pixel-wise directionality map was generated. We analyzed fiber trend by measuring the mean angles and quantified dispersion by calculating the standard deviation of the fiber direction over a region of 400 ?m x 400 ?m.

For the initial four samples, our analysis confirms a circumferential fiber pattern in the outer region of slices at all depths. We found that the standard deviation close to internal os showed no significance to the standard deviation close to external os ($p>0.05$), indicating comparable dispersion.

10053-44, Session 7

Correlation between polarization sensitive optical coherence tomography and SHG microscopy in articular cartilage

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Polarization-sensitive optical coherence tomography (PS-OCT) and second harmonic generation (SHG) microscopy are two imaging modalities both capable of analyzing the collagen fibers. Correlation between them has been discussed in skin samples, however, the potential of PS-OCT assessing microstructural features is not fully explored due to the random collagen orientation in skin. Articular cartilage, with abundant structural collagen fibers, is a suitable sample to further study the correlation between PS-OCT and SHG microscopy. Anatomical studies show that multilayer architecture of articular cartilage can be divided into four zones from its natural surface to the subchondral bone: the superficial zone, the middle zone, the deep zone, and the calcified zone. Different zones have different fiber orientations, which may cause different slope in cumulative phase retardation. Several groups have utilized PS-OCT phase retardation measurement to analyze the major collagen fiber orientation in articular cartilage. However, their works mainly focus on the global feature of articular cartilage, without considering its multi-layer structure. In this study, we use articular cartilage as a natural tissue model to investigate the contrast mechanism of PS-OCT in collagenous tissues with different fiber orientations. The phase retardation slope is quantified for the different layers of cartilage. The microscopic collagen fiber orientation in the different layers is identified by SHG imaging of the same cartilage tissue. By comparing the PS-OCT and SHG imaging, we demonstrate the relation between the micro-structural features with the quantitative analysis of PS-OCT phase retardation images. The potential of using PS-OCT to unveil the features beyond its resolution is evaluated. More quantitative analysis can be conducted in the further, including different illumination angles, and detection of the optic axis and local birefringence. The application can also be applied to other tissue types with abundant collagen fibers.

10053-45, Session 7

Parallel detection of Jones-matrix elements for polarization-sensitive OCT

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The polarization property of the sample can be characterized through Jones matrix. However, since four complex signals are required to measure the Jones matrix, the PS-OCT interferometers have had some drawbacks. In the case of frequency multiplexing and depth encoding, one of the drawbacks is a limited axial measurement range. With the progress of ultrahigh-speed and long-coherence-length swept sources, however, the previous approaches are not optimal for some applications, such as imaging of the anterior eye segment, where long axial measurement range of more than 10 mm is important.

Here, we present a parallel-detection approach that maintains the same axial measurement range as conventional OCT. Most of the interferometer consists of polarization-maintaining fiber-optic components that are effective for easy and stable alignment. The four Jones-matrix elements are detected by four balanced receivers individually, and digitized by two two-channel digitizers. This system has an axial measurement range of 15.51 mm, which is same as conventional OCT configuration.

The parallel-detection PS-OCT is also effective for cost reduction of the system, because the same photoreceivers and digitizers can be shared with conventional OCT. We demonstrate a potential of the parallel-detection PS-OCT for Jones-matrix imaging of the anterior eye segment without decreasing the axial measurement range, and show that it is a promising approach for replacing the conventional OCT engine to the Jones-matrix PS-OCT engine.

10053-46, Session 7

Polarization-sensitive plug-in optical module for a Fourier-domain optical coherence tomography system

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In this manuscript we communicate a theoretical study on a plug-in optical module to be used within a Fourier-domain optical coherence tomography system (FD-OCT). The module can be inserted between the object under investigation and any single-mode fiber based FD-OCT imaging instrument, enabling the latter to carry out polarimetric measurements on the former. Similarly to our previous communication, this is an active module which requires two sequential steps to perform a polarimetric measurement. Alternating between the two steps is achieved by changing the value of the retardance produced by two electro-optic polarization modulators, which behave as a polarization state rotator. By combining the rotation of the polarization state with a projection against a linear polarizer it is possible to ensure that the polarimetric measurements are free from any undesirable polarization effects caused by the birefringence in the collecting fiber and diattenuation in the fiber-based couplers employed in the system. Unlike our previous work, though, this module adopts an in-line configuration, employing a Faraday rotator to ensure a non-reciprocal behavior between the forward and backward propagation paths.

The module design also allows higher imaging rates due to the use of fast electro-optic modulators. Simulations have been carried out accounting for the chromatic effects of the polarization components, in order to evaluate the theoretical performance of the module.

10053-47, Session 7

Birefringence phantoms for polarization sensitive optical coherence tomography

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Polarization sensitive optical coherence tomography (PS-OCT) is increasingly used in a range of applications, both in bench-top and catheter-based imaging configurations. Reconstruction of tissue birefringence is subject to many system and processing-dependent artifacts. However, methods for the calibration and validation of PS-OCT are missing. Here, we report on a method to fabricate tissue-like imaging phantoms exhibiting clearly defined regions with controllable amounts of birefringence. We employed the photoelastic effect to enable the generation of controllable amounts of stress-induced birefringence in rubber samples, verified with polarized light microscopy. Pigmented ink was added to liquid latex solution to mold and cure rubber bands with controlled backscattering and transparency. Differently stretched segments were embedded in a stress-free background matrix to generate clearly defined areas with high

birefringence contrast in an area of homogenous backscatter intensity. Arranged in planar geometry or on the outside of a glass capillary, the stretched rubber bands defined phantoms for bench-top and catheter-based imaging, respectively. Segmentation of the defined regions of interest in the reconstructed volumetric birefringence tomograms enabled assessing measurement consistency, between repeated imaging with a single system, or between independent imaging systems.

Consistent and durable imaging phantoms are crucial for advancing PS-OCT imaging technology. Our tissue-like imaging phantoms exhibit clearly defined regions with controlled amounts of birefringence and facilitate testing, calibration, and validation of imaging systems and reconstruction strategies.

10053-48, Session 7

High sensitivity contrast enhanced optical coherence tomography for functional in vivo imaging

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Optical Coherence Tomography (OCT) enables real-time imaging of living tissues at cell-scale resolution over millimeters in three dimensions. Despite these advantages, functional biological studies with OCT have been limited by a lack of exogenous contrast agents that can be distinguished from tissue. Here we report an approach to functional OCT imaging that implements custom algorithms to spectrally identify unique contrast agents: large gold nanorods (LGNRs). LGNRs exhibit 110-fold greater spectral signal per particle than conventional GNRs, which enables detection of individual LGNRs in water and concentrations as low as 250 pM in the circulation of living mice. This translates to ~40 particles per imaging voxel in vivo. Unlike previous implementations of OCT spectral detection, the methods described herein adaptively compensate for depth and processing artifacts on a per sample basis. Collectively, these methods enable high-quality noninvasive contrast-enhanced imaging of OCT in living subjects, including detection of tumor microvasculature at twice the depth achievable with conventional OCT. Additionally, multiplexed detection of spectrally-distinct LGNRs was demonstrated to observe discrete patterns of lymphatic drainage and identify individual lymphangions and lymphatic valve functional states. These capabilities provide a powerful platform for molecular imaging and characterization of tissue noninvasively at cellular resolution, called MOZART.

10053-49, Session 8

A smart brain biopsy needle integrating an OCT needle probe with automated blood vessel detection in human patients

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Brain biopsies are a common neurosurgical technique to provide diagnosis of brain pathologies. However, they have a 2 - 3% risk of causing a significant hemorrhage, resulting in neurological deficit or death. We present a miniaturized OCT imaging probe for the detection of blood vessels, integrated into a Medtronic brain biopsy needle. The OCT needle consists of a length of single-mode fiber, terminated with spliced sections of coreless silica fiber and graded-index fiber to focus the light beam. This is terminated with angle-polished coreless silica fiber, using total internal reflection to redirect light perpendicular to the needle axis. The focusing optics are optimized to produce a light beam with a long working distance, with a FWHM lateral resolution of 42μm at 1.9mm from the probe. This optical assembly is encased in a stainless steel tube (OD 1.6mm) with a 1mm imaging window near the distal end. The OCT needle was then integrated with a Medtronic brain biopsy needle and connected to a ThorLabs Telesto II spectral-domain OCT system. A speckle decorrelation algorithm was implemented to automatically detect blood vessels, providing interactive feedback to the clinician during needle insertion. The blood vessel detection algorithm was incorporated into custom acquisition and reconstruction software. In vivo human measurements acquired during craniotomies of two patients to demonstrate the potential of the OCT needle probe to differentiate blood vessels from other tissue. These results demonstrate the first use of an OCT needle probe in human brains to identify blood vessels.

10053-50, Session 8

Intra-operative mapping of glioma infiltration in vivo in patients using OCT

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Glioma is the most common and aggressive adult primary brain cancer, with inevitable recurrence and limited survival times. Current literature has shown that maximal safe resection of the brain cancer can lead to improved overall survival and delayed recurrence. While some intra-operative imaging tools are already available (including MRI, CT, ultrasound, and fluorescence), these modalities have limitations in the ability to provide quantitative, real-time and/or continuous three-dimensional guidance in the operating room with optimal resolution and contrast. In a recent publication, we demonstrated the exciting potential of OCT in distinguishing ex vivo human brain cancer from non-cancer in 32 patients, with 92-100% sensitivity and 80-100% specificity. In addition, we have demonstrated a novel method to quantitatively analyze OCT data and to generate a color-coded optical property map for real-time, continuous guidance in brain cancer resections. While we have previously demonstrated the feasibility of this novel method in vivo in mice, the ultimate goal is to test OCT's ability in detecting infiltrated brain cancer margins in real-time, and in vivo in patients during surgery. This summary describes our most recent results in vivo in patients, as part of an international collaboration between Johns Hopkins Hospital in USA and Hospital Civil de Guadalajara in Mexico. To this date, we have collected OCT data from a total of 50 tissue samples from 8 patients with brain lesions. Our dataset include 4 glioma patients, 1 medulloblastoma patient, 2 patients with metastatic cancer to the brain and 1 patient with a non-cancer brain lesion.

10053-51, Session 8

Clinical assessment of human breast cancer margins with wide-field optical coherence micro-elastography

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Breast cancer has the second highest mortality rate of all cancers in females. Surgical excision of malignant tissue forms a central component of breast-conserving surgery (BCS) procedures. Incomplete excision of malignant tissue is a major issue in BCS with typically 20 – 30% cases requiring a second surgical procedure due to postoperative detection of tumor in the margin. A major challenge for surgeons during BCS is the lack of effective tools to assess the surgical margin intraoperatively. Such tools would enable the surgeon to more effectively remove all tumor during the initial surgery, hence reducing re-excision rates.

We report advances in the development of a new tool, optical coherence micro-elastography, which forms images, known as elastograms, based on mechanical contrast within the tissue. We demonstrate the potential of this technique to increase contrast between malignant tumor and healthy stroma in elastograms over OCT images. We demonstrate a key advance toward clinical translation by conducting wide-field imaging in intraoperative time frames with a wide-field scanning system, acquiring mosaicked elastograms with overall dimensions of ~50 ? 50 mm, large enough to image an entire face of most lumpectomy specimens. We describe this wide-field imaging system, and demonstrate its operation by presenting wide-field optical coherence tomography images and elastograms of a tissue mimicking silicone phantom and a number of representative freshly excised human breast specimens. Our results demonstrate the feasibility of scanning large areas of lumpectomies, which is an important step towards practical intraoperative margin assessment.

10053-52, Session 8

Extracting relevant information for cancer diagnosis from dynamic full field OCT through image processing and learning

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For a large number of cancer surgeries, the lack of reliable intraoperative diagnosis leads to reoperations or bad outcomes for the patients. To deliver better diagnosis, we developed Dynamic Full Field OCT (D-FFOCT) as a complement to FFOCT. FFOCT already presents interesting results for cancer diagnosis like Mohs surgery and reaching 96% accuracy on prostate cancer. D-FFOCT accesses the dynamic processes of metabolism and give new tools to diagnose the state of a tissue at the cellular level to complement FFOCT contrast. We developed a processing framework dedicated to maximize the information provided by the FFOCT technology as well as D-FFOCT and to synthetize a meaningful image. We use different time processing to generate metrics (standard deviation of time signals, decorrelation times and more) and spatial processing to sort out structures and the corresponding imaging modality which is the most appropriate. Sorting was achieved through quadratic discriminant analysis in a N-dimension parametric space corresponding to our metrics. Combining the best imaging modalities for each structure leads us to a rich morphology image. This image displaying the morphology is then colored to represent the dynamic behavior of these structures (slow or fast). Therefore, we achieved a micron resolved image, rich of both FFOCT ability of imaging fixed and highly backscattering structures as well as D-FFOCT ability of imaging low level scattering cellular level details. We believe that this morphological contrast close to histology and the dynamic behavior contrast will push forward the limits of intraoperative diagnosis further on.

10053-53, Session 8

Ex vivo and in vivo label-free imaging of lymphatic vessels using OCT lymphangiography

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We have been developing an automated method to image lymphatic vessels both ex vivo and in vivo with optical coherence tomography (OCT), using their optical transparency. Our method compensates for the OCT signal attenuation for each A-scan in combination with the correction of the confocal function and sensitivity fall-off, enabling reliable thresholding of lymphatic vessels from the OCT scans. Morphological image processing with a segment-joining algorithm is also incorporated into the method to mitigate partial-volume artifacts, which are particularly evident with small lymphatic vessels. Our method is demonstrated for two different clinical application goals: the monitoring of conjunctival lymphatics for surgical guidance and assessment of glaucoma treatment; and the longitudinal monitoring of human burn scars undergoing laser ablation treatment. We present examples of OCT lymphangiography ex vivo on porcine conjunctivas and in vivo on human burn scars, showing the visualization of the lymphatic vessel network and their longitudinal changes due to treatment.

10053-54, Session 8

Clinical potential of wide-field OCT angiography for monitoring human oral cavity lesions in vivo

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We present one applicability of OCT angiography (OCTA) for monitoring wide-field human oral cavity lesions. Three-dimensional (3D) structure and vasculature images of labial mucosa tissue are obtained at a single 3D acquisition, covering a field of view (FOV) of 10 ? 10 mm2. Two pathologic mucosal sites with ulcer wounds are located and examined from simultaneously acquired wide-field OCT tomograms and angiograms, which are further applied to monitor the wound healing process of the lesions over two weeks. For quantification of the wound healing, the capillary loop density within the lamina propria layer are evaluated on OCT angiograms for each examination, indicating statistical differences between healthy and diseased vascular conditions over days. Furthermore, tissue anatomy and vessel morphology of other ulcer susceptible sites such as tongue, alveolar mucosa and labial frenulum, are also imaged to validate promise of the proposed method as a clinical tool for diagnosis of oral tissue abnormalities.

10053-55, Session 8

Automated classification and diagnosis of otitis media using OCT

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Our group has developed handheld probes and portable systems for OCT imaging that have been used in clinical studies to observe and characterize various presentations of ear infections (otitis media, OM) in the physician's office, specialist's clinic, and even intraoperatively in the surgical ward under various IRB approved studies. A wide variety of OCT images characterizing physiological changes in the TM and middle ear space have been collected and strongly associated with different middle ear infection states. Although OCT data provides quantitative information about these infection-related changes, image-based data still must be interpreted and analyzed by a trained expert reader to extract relevant clinical information. To date, no OCT-based metrics or guidelines exist to stratify the many different clinical presentations of OM.

In this work, an automated machine learning-based technique will be developed and applied to classify morphological changes in OCT images of tissue associated with various stages of OM infection. A random-forest based classifier is utilized with features based on various statistics and metrics derived from OCT B-scans and aimed towards quantifying the structural changes of the TM and middle ear. Classifier accuracy was estimated using randomized 10-fold cross validation, which yielded a classification accuracy of 81%. Further development and testing of this automated classification algorithm will provide a framework for an untrained user to collect OCT data and receive a diagnostic prediction for the presence and type of OM. Similarly, these techniques provide evidence that OCT data may be a useful tool for the clinical management of specific chronic ear diseases.

10053-56, Session 8

Seeing is believing: real-time visualization and interaction with optical coherence tomography volumes in immersive virtual reality

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Virtual reality (VR) head-mounted displays are an attractive method for displaying intrasurgical optical coherence tomography (OCT) volumes as evidenced by existing commercial and research efforts. Compared with current 3D TV and heads-up display (HUD) VR systems, head-mounted displays (HMDs) provide a much larger field of view while allowing immersed users to move through space. In the ophthalmic surgery context, we believe immersed surgeons can gain a heightened situational awareness through careful context-dependent feedthrough of relevant information, including patient vital signs, OCT visualizations, and operating room video feeds. Furthermore, with an intuitive VR interface design, an HMD-immersed surgeon has access to much more information than they could see through the microscope oculars alone. As an example of VR HMDs' potential, we demonstrate real-time inter-active viewing of OCT volumes in a commercial HTC Vive(R) immersive VR system. Our VR rendering pipeline is built using OpenGL and OpenVR and supports interactive translation, rotation, and scaling of volumes using the Vive controllers. We use previously reported ray casting techniques with modifications to support an arbitrary pro-jection matrix and clip bounds for OCT volumetric rendering and then composite the volumetric render image into a standard 3D scene with optimizations to preserve volume sharpness. Our pipeline is capable of displaying a sam-ple volume at the Vive's native screen refresh rate of 90 Hz with stereo volume ray casting at 45 Hz for a 512 by 512 pixels volumetric render. We believe VR OCT volume displays will advance ophthalmic surgery towards VR-integrated surgery.

10053-57, Session 9

Depth of field extension by annular phase plate for optical coherence tomography endoscopy

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High-resolution endoscopic imaging can provide a powerful diagnostic tool for physicians for early diagnosis of cancer in gastrointestinal- and respiratory systems or diagnosis of cardiovascular diseases such as atherosclerotic plaques. Swept source Optical Coherence Tomography (OCT) endoscopes enable in vivo, volumetric imaging of biological tissue. To overcome the current trade-off in OCT between lateral resolution and the depth over which a good lateral resolution (i.e. the focus) is maintained, an annular phase plate is used as a synthetic aperture to depth-encode the light in reflection. This enables compensation of the wave front curvature of light in reflection from defocused locations, which is done in post processing and can lead to more than a fourfold increase of high resolution imaging depth (Rayleigh range). This refocusing is done in three consecutive steps: depth-decoding the images by applying a wavenumber dependent phase, correcting for an overall wavenumber independent phase caused by the phase plate and correcting for the defocus phase, i.e. the wave front curvature. In this study we demonstrate a macroscopic version of a focus-extended endoscope, in which the phase factors needed to refocus are constant for each measurement and therefore lead to a faster and more reliable refocusing process compared to the previous setup. Refocused images of a phantom of melamine beads in a silicone matrix are shown to demonstrate the capabilities of the current setup. These results show a promising increase of high resolution imaging depth, and the next step towards a high resolution imaging OCT endoscope.

10053-58, Session 9

A coaxially focused multi-mode (CAFM) beam for extended depth of focus optical coherence tomography

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Conventional OCT images, obtained using a focused Gaussian beam have a lateral resolution of approximately 30 ?m and a depth of focus (DOF) of 2-3 mm, defined as the confocal parameter (twice of Gaussian beam Rayleigh range). Improvement of lateral resolution without sacrificing imaging range requires techniques that can extend the DOF. Previously, we described a self-imaging wavefront division optical system that provided an estimated one order of magnitude DOF extension. In this study, we further investigate the properties of the coaxially focused multi-mode (CAFM) beam created by this self-imaging wavefront division optical system and demonstrate its feasibility for real-time biological tissue imaging. Gaussian beam and CAFM beam fiber optic probes with similar numerical apertures (objective NA≈0.5) were fabricated, providing lateral resolutions of approximately 2 ?m. Rigorous lateral resolution characterization over depth was performed for both probes. The CAFM beam probe was found to be able to provide a DOF that was approximately one order of magnitude greater than that

of Gaussian beam probe. By incorporating the CAFM beam fiber optic probe into a ?OCT system with ~1.5 ?m axial resolution, we were able to acquire cross-sectional images of swine small intestine ex vivo, enabling the visualization of subcellular structures, providing high quality OCT images over more than a 300 ?m depth range.

10053-59, Session 9

Scattering angle resolved optical coherence tomography for in vivo murine retinal imaging

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Optical coherence tomography (OCT) retinal imaging contributes more each year to understanding central nervous system (CNS) diseases because the eye serves as a "window to the brain" with direct optical access to nonmyelinated retinal ganglion cells. Many CNS diseases are associated with neuronal changes beyond the resolution of standard OCT retinal imaging systems. Though studies have shown the utility of scattering angle resolved (SAR) OCT for particle sizing and detecting disease states ex-vivo, a compact SAR-OCT system for rodent retinal imaging has not previously been reported. We have constructed a compact, fiber-based SAR-OCT system (swept source at 1310 nm ± 63 nm, 100 kHz scan rate) for mouse retinal imaging with a partial glass window (center aperture) for angular discrimination of backscattered light. The proposed SAR-OCT design provides a contrast mechanism able to detect neuronal changes in the central nervous system that are beyond the resolution of standard OCT retinal imaging methods. The reported SAR-OCT design incorporates a dual axis MEMS mirror conjugate to the ocular pupil plane. Pivoting at the pupil in two dimensions limits optical aberrations and vignetting while maximizing angular discrimination and collection efficiency with a high numerical aperture objective (f=10 mm, NA=0.45). The laser's 100 kHz scan rate enhances OCT angiography techniques augmenting the SAR-OCT system's diagnostic power. Mouse retinas are imaged using SAR-OCT in longitudinal mouse studies to demonstrate advantages of the proposed design over previous rodent retinal OCT systems.

10053-60, Session 9

Line-field time domain OCT using off-axis spatial signal modulation with digital refocusing

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We present a line field time domain OCT using off-axis modulation with digital adaptive optics. Time domain en-face OCT has the advantage of high speed en-face imaging guaranteeing good phase correlation across the recorded sample. It has recently been shown that the high lateral phase correlation in en-face time domain OCT allows for digital aberration correction of retinal OCT images in vivo. En-face time domain OCT needs reference arm modulation in order to sample the complex OCT signal. This has been achieved either by mechanical piezoelectric actuators, electro-optic or acousto-optic modulators. In the present work we demonstrate a simple method based on line field OCT using an off-axis reference arm configuration which introduces spatial modulation. This carrier frequency is used to filter out the complex OCT signal after spatial Fourier transform. The advantage of this configuration is the intrinsic higher speed due to parallel recording together with a slightly higher sensitivity by the higher exposure limits of an extended field illumination. The increased speed leads to a better phase correlation allowing for digital phase corrections. We introduce the principle and demonstrate first in vitro results. The system achieves up to 100 Hz en-face rate. Employing a swept source we can tune the coherence gate-width by changing the exposure time of the line-sensor. We further show the ability to digitally refocus the en-face images.

10053-61, Session 9

In vivo full-field time-domain optical coherence tomography using a spatially coherent off-axis reference

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Time domain OCT measures the interference between sample and reference radiation as a function of the reference arm length. In full-field-OCT (FF-OCT) a camera is used instead of a scanned beam for a parallel detection of the interference pattern and thus acquiring a complete en face image. Because multiple images have to be acquired to resolve the phase ambiguity, this method is prone to motion artifacts.

We present a novel motion-insensitive approach to FF-OCT. Spatially coherent illumination and an off-axis reference beam is used to introduce path-length differences between reference and sample light in neighboring pixels. This spatial carrier frequency replaces the temporal carrier frequency in scanned TD-OCT.

The setup is based on a Mach-Zehnder interferometer with a super-luminescent diode and a CMOS area camera. The Sensitivity of the system was determined to be 75 dB. The field of view was 1.42 x 1.42 mm. Each frame had 237x237 lateral channels at an axial resolution of 9 μm in tissue. By step-wise changing the length of the reference arm between the en face scans, volumetric in vivo FF-OCT measurements of the human retina have been acquired within 1.3 s.

OCT with a spatially coherent off-axis reference beam is suitable for in vivo imaging of human retina. The quality of the images is sufficient to discriminate the different tissue layers.

10053-62, Session 9

Master/slave: the ideal tool for coherence revival based optical coherence tomography imaging instruments

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Currently, optical coherence tomography (OCT) is almost exclusively implemented via Fast Fourier Transforms (FFTs) in both, camera (CB) or swept source (SS) based OCT instruments. Here, we demonstrate that the Master/Slave (MS) method of implementing spectral (Fourier) domain OCT can be an useful alternative to the FFT based method, especially when combined with the coherence revival (CR) technique. The MS method

eliminates several drawbacks associated to the FFT technique, two relevant for the CR technique. As the laser cavity length is combined with the optical path difference in the interferometer, the combined results is a highly dispersive interference. This leads to a chirp modulation of the channeled spectrum with a high frequency. The CR technique demands therefore good resampling of data to counteract the dispersive modulation as well as its nonlinearity due to the CR typical modulation. The total tolerance of the MS method to nonlinear tuning as well as to dispersion in the combined interferometer, makes the MS ideally suited to the CR practice. Additionally, enhanced versatility is brought about by the MS method in displaying shorter axial range images than that determined by the digital sampling of the data when using the FFT. This brings an immediate improvement in the speed of displaying cross-sectional images at high rates without the need of extra hardware such as graphics processing units or field programmable gate arrays.

10053-63, Session 10

High-sensitivity supercontinuum-based parallel line-field optical coherence tomography with 1 million A-lines/s

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While traditional, flying-spot, spectral domain OCT systems can achieve MHz linerates, they are limited by the need for mechanical scanning to produce a B-mode image. Line-field OCT (LF OCT) removes the need for mechanical scanning by simultaneously recording all A-lines on a 2D CMOS sensor. Our LF OCT system operates at the highest A-line rate of any spectral domain (SD) LF OCT system reported to date (1,024,000 A-lines/s). This is comparable with the fastest flying-spot SDOCT system reported. Additionally, all OCT systems face a tradeoff between imaging speed and sensitivity. Long exposure times improve sensitivity but can lead to undesirable motion artifacts. LF OCT has the potential to relax this tradeoff between sensitivity and imaging speed because all A-lines are exposed during the entire frame acquisition time. However, this advantage has not yet been realized due to the loss of power-per-A-line by spreading the illumination light across all A-lines on the sample. Here we use a supercontinuum source to illuminate the sample with 500mW of light in the 605-950 nm wavelength band, effectively providing 480 μW of power-per-A-line, with axial and lateral resolutions of 1.8 μm and 14 μm, respectively. With this system we achieve the highest reported sensitivity (113 dB) of any LF OCT system. We then demonstrate the capability of this system by capturing the rapidly beating cilia of human bronchial-epithelial cells in vitro. The combination of high speed and high sensitivity offered by supercontinuum-based LF SD OCT offers new opportunities for studying cell and tissue dynamics.

10053-64, Session 10

Analysis of FDML lasers with meter range coherence

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FDML lasers are known to provide sweep rates in the MHz range at high wide optical bandwidths, making them ideal sources for high speed optical coherence tomography OCT. Recently, at lower speed, ultralong-range swept-source optical coherence tomography has been demonstrated, enabled by recent developments of new vertical cavity surface emitting lasers (VCSEL), providing high sweep rates and meter range coherence lengths. Concerning the coherence, common FDML lasers until now have not achieved comparable performance. We developed an extremely well dispersion compensated FDML laser, running at 3.2 MHz sweep rate and 120 nm spectral bandwidth. Using a 70 GHz real time oscilloscope we have investigated the causes of high frequency noise in FDML lasers and demonstrate that they can be vastly reduced by compensating the lasers cavity dispersion with very high precision. We then demonstrate that the new laser offers dramatically improved noise characteristics as well as meter class instantaneous coherence properties. To demonstrate the immediate benefit of our development for OCT, we employed the laser into a live 4D-OCT system running at 22 volumes per second. As a result, we can demonstrate high quality 4D-OCT videos with imaging ranges of up to 25 mm.

10053-65, Session 10

High density B-scans using a MHz FDML swept laser source extends the dynamic range of Doppler OCT and improves image contrast

Sahar Elahi, Lars Thrane, Andrew M. Rollins, Michael W. Jenkins, Case Western Reserve Univ. (United States)

The limited dynamic range of optical coherence tomography (OCT) Doppler velocity measurements makes it difficult to conduct experiments on samples requiring a large dynamic range without phase wrapping at high velocities or loss of sensitivity at slow velocities. Hemodynamics and wall motion undergo significant increases in velocity as the embryonic heart develops. Experimental studies indicate that altered hemodynamics in early-stage embryonic hearts can lead to congenital heart diseases (CHDs), motivating close monitoring of blood flow over several stages of development. We have built a high-speed OCT system using an FDML laser (Optores GmbH, Germany) at a sweep rate of 1.68 MHz (axial resolution - 12 ?m, sensitivity - 105 dB, phase stability - 17 mrad). The speed of this OCT system allows us to acquire high-density B-scans to obtain an extended velocity dynamic range without sacrificing the frame rate (100 Hz). The extended dynamic range within a frame is achieved by varying the A-scan interval at which the phase difference is found, enabling detection of velocities ranging from tens of microns per second to hundreds of millimeters per second. The extra lines in a frame can also be utilized to improve the structural and Doppler images via complex averaging. In structural images where the presence of blood causes additional scattering, complex averaging helps retrieve features located deeper in the tissue. Moreover, high-density frames can be registered to 4D volumes to determine the orthogonal direction of flow for calculating shear stress as well as estimating the cardiac output. In conclusion, high density B-scans acquired by our high-speed OCT system enable image enhancement and direct measurement of biological parameters in cohort studies.

10053-66, Session 10

High-resolution and deep-tissue imaging with full-range, ultrahigh-resolution spectral-domain optical coherence tomography in 1.7 um wavelength region

Hiroyuki Kawagoe, Masahito Yamanaka, Nagoya Univ. (Japan); Shuichi Makita, Yoshiaki Yasuno, Univ. of Tsukuba (Japan); Norihiko Nishizawa, Nagoya Univ. (Japan)

We developed full-range, ultrahigh-resolution (UHR) spectral-domain optical coherence tomography (SD-OCT) in 1.7 um wavelength region for high-resolution and deep-penetration OCT imaging of turbid tissues. To realize an ultrahigh axial resolution, the ultra-broadband supercontinuum source at 1.7 um wavelength with a spectral width of 0.4 um at FWHM and home-built spectrometer with a detection range from 1.4 to 2.0 um were employed. Consequently, we achieved the axial resolution of 3.6 um in tissue (a refractive index n = 1.38). To observe deep regions of turbid tissues while keeping the ultrahigh axial resolution, a full-range OCT method to eliminate a coherent ghost image was utilized for our UHR-SD-OCT. Because the full-range method allows us to avoid the formation of a coherent ghost

image when the zero delay position is in the inside of specimens, we set the zero delay position to the laser focus position in this study, and then, a region of interest in specimens was moved to the laser focus position where the highest signal intensity is achieved, resulting in the improvement of the observation depth. Thanks to the deep-penetration property of the 1.7 um light and elimination of a ghost image, we successfully demonstrated the visualization of the mouse brain structures at a depth over 1.5 mm from the surface with the 1.7 um UHR-SD-OCT. In this experiment, we confirmed that the brain specific structures, such as corpus callosum, pyramidal cell layer, and hippocampus, were clearly observed.

10053-67, Session 10

Ultralong-range optical coherence tomography-based angiography by akinetic swept source

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Optical coherence tomography (OCT) based angiography (OCTA) has increasingly become an important inspection tool due to its capability of high-speed, non-invasive, volumetric in vivo imaging of both structure and vasculature at micron-scale resolution. However, the picturing for most current OCTA systems is strictly limited within a small field of view (FOV) where the blood vessels are located in the superficial layer of a flat tissue due to a relatively short imaging range (typically ~2-6 mm) and significant sensitivity roll-off at long depth position. To overcome this challenge, we demonstrate an ultralong-range OCT system for vascular imaging based on an akinetic swept source. The single mode operation of the light source and the high-speed detection in the system enable us to achieve up to 46 mm long imaging range with unprecedented roll-off performance. To demonstrate the advantage of the ultralong-range SS-OCT system, a comparison of in vivo vascular imaging of the entire mice brain with traditional SD-OCT system was presented. Additionally, the blood flow images at different depth positions are captured by this system. This ultralong-range OCTA system demonstrates better imaging performance to visualize the vascular network for large FOV without the restriction of flatness and depth position. This work is expected to benefit the current clinical practice and bring new opportunities for OCTA in the field of diagnosis, treatment and monitoring.

10053-68, Session 10

Optical coherence microscopy in 1700-nm spectral band for high-resolution deep-tissue imaging

Masahito Yamanaka, Tatsuhiro Teranishi, Hiroyuki Kawagoe, Norihiko Nishizawa, Nagoya Univ. (Japan)

Optical coherence microscopy (OCM) is a high-resolution imaging technique based on optical coherence tomography and confocal microscopy. The recent studies on OCM operating at 800-1300 nm spectral region have shown that OCM enables to visualize micrometer- or sub-micrometer-scale structures of animal tissues. Although OCMs offers such high-resolution label-free imaging capability of animal tissues, the imaging depth was restricted by multiple light scattering and light absorption of water in samples. Here, for high-resolution deep-tissue imaging, we developed an OCM in the 1700-nm spectral band by using a supercontinuum (SC) source with a Gaussian-like spectral shape in the wavelength region. Recently, it has been reported that the 1700-nm spectral band is a promising choice for enhancing the imaging depth in the observation of turbid scattering tissues because of the low attenuation coefficient of light. In this study, to clarify that the 1700-nm OCM has a potential to realize the enhanced imaging depth, we compared the attenuation of the signal-to-noise ratio between the 1700-nm and 1300-nm OCM imaging of a mouse brain under the same signal detection sensitivity condition. The result shows that the 1700-nm OCM enables us to achieve the enhanced imaging depth. In this 1700-nm OCM, we also confirmed that the lateral resolution of 1.3 μm and axial resolution of 2.8 μm in tissue were achieved.

10053-69, Session 11

Non-contact rapid optical coherence elastography by high-speed 4D imaging of elastic waves

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Shear wave OCE (SW-OCE) uses an OCT system to track propagating mechanical waves, providing the information needed to map the elasticity of the target sample. In this study we demonstrate high speed, 4D imaging to capture transient mechanical wave propagation. Using a high-speed Fourier domain mode-locked (FDML) swept-source OCT (SS-OCT) system operating at ~1.62 MHz A-line rate, the equivalent volume rate of mechanical wave imaging is 16 kvps (kilo-volumes per second), and total imaging time for a 6 x 6 x 3 mm volume is only 0.32 s. With a displacement sensitivity of ~10 nanometers, the proposed 4D imaging technique provides sufficient temporal and spatial resolution for real-time optical coherence elastography (OCE). Combined with a new air-coupled, high-frequency focused ultrasound stimulator requiring no contact or coupling media, this near real-time system can provide quantitative information on localized viscoelastic properties. SW-OCE measurements are demonstrated on tissue-mimicking phantoms and porcine cornea under various intra-ocular pressures. In addition, elasticity anisotropy in the cornea is observed. Images of the mechanical wave group velocity, which correlates with tissue elasticity, show velocities ranging from 4-30 m/sec depending on pressure and propagation direction. These initial results strong suggest that 4D imaging for real-time OCE may enable high-resolution quantitative mapping of tissue biomechanical properties in clinical applications.

10053-70, Session 11

Line-field low coherence holography for ultra-fast assessment of tissue biomechanical properties

Chih-Hao Liu, Alexander Schill, Manmohan Singh, Chen Wu, Raksha Raghunathan, Kirill V. Larin, Univ. of Houston (United States)

Changes in the biomechanical properties of tissues are often associated with disease etiology and can provide quantitative information for clinical diagnosis. Tissue elasticity is often assessed by analyzing the speed of an elastic wave, such as in supersonic shear wave imaging and magnetic resonance elastography techniques. However, insufficient spatial resolution and large stimulation forces limit their application in small samples (dimensions on the order of millimeters or micrometers). Optical coherence elastography (OCE) is an emerging technique that provides local biomechanical properties with micrometer scale resolution. However, conventional point-by-point scanning OCE methods require long acquisition times (tens of seconds) that are unfeasible for clinical use due to motion artifacts, and repeated external excitations. Here, we demonstrate a noncontact ultrafast line-field low coherent holography system (LF-LCH) integrated with spatial phase shifting algorithm for phase retrieval based on a single interferogram. The proposed method using the Hilbert transform outperforms the Fourier transform-based technique in LF-LCH. Spatio-temporal maps of elastic wave propagation were acquired using a single air-pulse excitation and the acquisition speed can be optimized to

less than 10 ms. Results on homogenous, transversely heterogeneous agar phantoms and ex vivo chicken breast agreed well with mechanical testing, demonstrating that this method can accurately detect tissue stiffness with an ultrafast line rate of 200 kHz using a robust phase retrieval algorithm, which is among the highest speed for lateral imaging of elastic wave propagation with optical elastography methods.

10053-71, Session 11

Viscosity measurement in microliter samples using phase-sensitive OCT

Brecken J. Blackburn, Shi Gu, Michael W. Jenkins, Andrew M. Rollins, Case Western Reserve Univ. (United States)

Viscosity is often a critical characteristic of biological fluids such as blood and mucus. However, traditional rheology is often inadequate when only small quantities of sample are available. A robust method to measure viscosity of microquantities of biological samples could lead to a better understanding and diagnosis of diseases. Here, we present a method to measure viscosity by observing particle Brownian motion within a sample. M-mode optical coherence tomography (OCT) imaging, obtained with a phase-sensitive 47 kHz spectral domain system, yields a viscosity measurement from multiple 200-1000 microsecond frames. This very short period of continuous acquisition, as compared to laser speckle decorrelation, decreases sensitivity to bulk motion, thereby potentially enabling in vivo and in situ applications. The theory linking g(1) first-order image autocorrelation to viscosity is derived from first principles of Brownian motion and the Stokes-Einstein relation. To improve precision, multiple windows acquired over 500 milliseconds are analyzed and the resulting linear fit parameters are averaged. Verification experiments were performed with 200 μL samples of glycerol and water with polystyrene microbeads. Lateral bulk motion up to 2 mm/s was tolerated and accurate viscosity measurements were obtained to a depth of 400 μm or more. Additionally, the method measured a significant decrease of the apparent diffusion constant of soft tissue after formalin fixation, suggesting potential for mapping tissue stiffness over a volume.

10053-72, Session 11

Quantified elasticity mapping of ocular tissue using acoustic radiation force optical coherence elastography

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Age-related macular degeneration and keratoconus are two ocular diseases occurring in the posterior and anterior eye, respectively. In both conditions, the mechanical elasticity of the respective tissues changes during the early onset of disease. It is necessary to detect these differences and treat the diseases in their early stages to provide proper treatment. Acoustic radiation force optical coherence elastography is a method of elasticity mapping using confocal ultrasound waves for excitation and Doppler optical coherence tomography for detection. We report on an ARF-OCE system that uses modulated compression wave based excitation signals, and detects the spatial and frequency responses of the tissue. First, all components of the system is synchronized and triggered such that the signal is consistent between frames. Next, phantom studies are performed to validate and calibrate the relationship between the resonance frequency and the Young's modulus. Then the frequency responses of the anterior and posterior eye are detected for porcine and rabbit eyes, and the results correlated to the elasticity. Finally, spatial elastograms are obtained for a porcine retina. Layer segmentation and analysis is performed and correlated to the histology of the retina, where five distinct layers are recognized. The elasticities of the tissue layers will be quantified according to the mean thickness and displacement response for the locations on the retina. This study is a stepping stone to future in-vivo animal studies, where the elastic modulus of the ocular tissue can be quantified and mapped out accordingly.

10053-73, Session 11

Model-independent quantification of soft tissue viscoelasticity with dynamic optical coherence elastography

Nichaluk Leartprapun, Steven G. Adie, Cornell Univ. (United States)

In order to obtain a comprehensive understanding of the mechanical properties of viscoelastic soft tissues for biomedical applications and mechanobiology research, both the elasticity and the viscosity must be probed. Although optical coherence elastography (OCE) has emerged as a promising tool for biological tissue mechanical characterization, quantitative OCE methods have mostly been limited to purely elastic characterization from shear wave speed or relied on the use of a presumed linear viscoelastic mechanical model. We present a model-independent reconstruction of viscoelastic complex shear modulus with dynamic acoustic radiation force OCE imaging of propagating shear wave. The real and imaginary parts of the complex shear wave number were directly extracted from the transverse phase evolution and amplitude attenuation profiles of the measured shear wave field, enabling the reconstruction of shear storage (G') and shear loss (G") moduli. Experimental demonstration in three viscoelastic tissue-mimicking phantoms highlights significant variation in the viscoelastic properties captured by the loss ratio, G"/G', that was not apparent from the shear wave speed measurements. Our results suggest that elasticity imaging based on shear wave speed alone could neglect potentially significant variations in the viscoelastic properties of tissues.

10053-74, Session 11

Measurement of time-varying displacement fields in cell culture for traction force optical coherence microscopy

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Mechanobiology is an emerging field which seeks to link mechanical forces and properties to the behaviors of cells and tissues in cancer, stem cell growth, and other processes. Traction force microscopy (TFM) is an imaging technique that enables the study of traction forces exerted by cells on their environment to migrate as well as sense and manipulate their surroundings. To date, TFM research has been performed using incoherent imaging modalities and, until recently, has been largely confined to the study of cell-induced tractions within two-dimensions using highly artificial and controlled environments. As the field of mechanobiology advances, and demand grows for research in physiologically relevant 3D culture and in vivo models, TFM will require imaging modalities that support such

settings. Optical coherence microscopy (OCM) is an interferometric imaging modality which enables 3D cellular resolution imaging in highly scattering environments. Moreover, optical coherence elastography (OCE) enables the measurement of tissue mechanical properties. OCE relies on the principle of measuring material deformations in response to artificially applied stress. By extension, similar techniques can enable the measurement of cell-induced deformations, imaged with OCM. We propose traction force optical coherence microscopy (TF-OCM) as a natural extension and partner to existing OCM and OCE methods. We report the first use of OCM data and digital image correlation to track temporally varying displacement fields exhibited within a 3D culture setting. These results mark the first steps toward the realization of TF-OCM in 2D and 3D settings, bolstering OCM as a platform for advancing research in mechanobiology.

10053-75, Session 11

Characterization of nonlinear elasticity for biological tissue using quantitative optical coherence elastography

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Optical coherence elastography (OCE), a functional extension of optical coherence tomography (OCT), measures the mechanical response (deformation, resonant frequency, elastic wave propagation) of biological tissue under mechanical stimulation to assess the mechanical properties. Compared to conventional technologies for mechanical characterization, OCE instrument can be miniaturized using fiber optic components. Therefore, OCE allows minimally invasive in vivo mechanical characterization of biological tissue and has great potential in various biomedical applications, such as the study of traumatic brain injury, cancer diagnosis and surgical guidance. However, conventional OCE techniques lack the mechanism to quantify the force exerted to tissue. This has significantly limited OCE's capability to accurately characterize the mechanical properties of biological tissue, because most biological tissues exhibit nonlinear elastic behavior. In our laboratory, we developed a quantitative OCE (qOCE) system that had a fiber optic probe with an integrated force sensor. For mechanical characterization, the qOCE probe is used to apply quasi-static indentation on the sample. Through Doppler analysis of signals acquired by the qOCE probe during the indentation process, the probe-tissue interaction force (F) and the apparent stress (?=Force/Area) were quantified. Similarly, tissue displacement (?d) and the apparent strain ?=?d/L0 were quantified. High-speed software based on graphic processing units (GPU) was developed to obtain stress-strain curve in real-time during the quasi-static indentation process. Using the qOCE system, we performed stress-strain measurement on silicone rubber phantom, in vivo human skin tissues and ex vivo rat brain tissues. Our results demonstrated the capability of our qOCE technology in characterizing nonlinear elasticity.

10053-76, Session 11

Ultrasensitive detection of nanoparticles using dual optical lock-In microscopy

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The aim of this study was to demonstrate an interferometric method for photothermal effect detection based on optical lock-in detection principle. The proposed approach was based on the detection of beating frequency of the optical signal that allows for maintaining high frequencies of photothermal effect modulation and high detection sensitivity. The OCM system allowing for additional excitation of nanoparticles was developed. We performed tests of our technique using well-defined nanoparticle samples, and optimized the imaging parameters to obtain the highest contrast. We also applied Dual Optical Lock-In method to image the samples with micrometer resolution.

10053-77, Session 12

Multi-volumetric registration and mosaicking using swept-source spectrally encoded scanning laser ophthalmoscopy and optical coherence tomography

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Ophthalmic diagnostic imaging using optical coherence tomography (OCT) is limited by bulk eye motions and a fundamental trade-off between field-of-view (FOV) and sampling density. Here, we introduced a novel multi-volumetric registration and mosaicking method using our previously described multimodal swept-source spectrally encoded scanning laser ophthalmoscopy and OCT (SS-SESLO-OCT) system. Our SS-SESLO-OCT acquires an entire en face fundus SESLO image simultaneously with every OCT cross-section at 200 frames-per-second. In vivo human retinal imaging was performed in a healthy volunteer, and three volumetric datasets were acquired with the volunteer moving freely and re-fixating between each acquisition. In post-processing, SESLO frames were used to estimate en face rotational and translational motions by registering every frame in all three volumetric datasets to the first frame in the first volume. OCT cross-sections were contrast-normalized and registered axially and rotationally across all volumes. Rotational and translational motions calculated from SESLO frames were applied to corresponding OCT B-scans to compensate for inter- and intra-B-scan bulk motions, and the three registered volumes were combined into a single interpolated multi-volumetric mosaic. Using complementary information from SESLO and OCT, we demonstrated multi-volumetric registration and mosaicking to recover regions of missing data resulting from blinks, saccades, and ocular drifts using mutual information from serially acquired volumes. We believe our registration method can be directly applied for multi-volumetric motion compensation, averaging, widefield mosaicking, and vascular mapping with potential applications in ophthalmic clinical diagnostics, handheld imaging, and intraoperative guidance.

10053-78, Session 12

Data-based online nonlinear extremum-seeker for wavefront sensorless adaptive optics OCT

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Adaptive optics has been successfully applied to cellular resolution imaging of the retina, enabling visualization of the characteristic mosaic patterns of the outer retina. Wavefront sensorless adaptive optics (WSAO) is a novel technique that facilitates high resolution ophthalmic imaging; it replaces the Hartmann-Shack Wavefront Sensor with an image-driven optimization algorithm and mitigates some the challenges encountered with sensor-based designs. However, WSAO generally requires longer time to perform aberrations correction than the conventional closed-loop adaptive optics. When used for in vivo retinal imaging applications, motion artifacts during the WSAO optimization process will affect the quality of

the aberration correction. A faster converging optimization scheme needs to be developed to account for rapid temporal variation of the wavefront and continuously apply corrections. In this project, we investigate the Databased Online Nonlinear Extremum-seeker (DONE), a novel non-linear multivariate optimization algorithm in combination with in vivo human WSAO OCT imaging. We also report both hardware and software updates of our compact lens based WSAO 1060nm swept source OCT human retinal imaging system, including real time retinal layer segmentation and tracking (ILM and RPE), hysteresis correction for the multi-actuator adaptive lens, precise synchronization control for the 200kHz laser source, and a zoom lens unit for rapid switching of the field of view. Cross sectional images of the retinal layers and en face images of the cone photoreceptor mosaic acquired in vivo from research volunteers before and after WSAO optimization are presented.

10053-79, Session 12

Optical mapping models of heterogeneous atria tissue informed by optical coherence tomography

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Optical mapping models are powerful tools for interpreting optical mapping experiments and exploring the electrophysiology of arrhythmias. However, atria optical mapping models are lacking, likely in part due to the difficulty of imaging structural detail in the atria. Optical coherence tomography (OCT), however, is well-suited for imaging atrial tissue, and we present the use of OCT to inform detailed tissue structure of atria optical mapping models.

Volumetric OCT image sets were acquired from swine and human atria. One of the modeled swine samples included an ablation lesion, while the modeled human atria sample contained regions of myocardium, collagen, and adipose tissue. Image stitching was performed on several overlapping OCT volumes to obtain a larger field of view. Fiber orientation, tissue geometry, and the varying tissue regions were extracted from OCT and incorporated into models for electrophysiological and photon scattering simulations, carried out in the multi-scale finite element package “Continuity 6” and Monte Carlo simulator “TIM-OS”, respectively. The two simulations were coupled to simulate the optical mapping signal in the tissue-specific models.

Influences of the tissue structure on the electrophysiology and optical mapping signals were observed. Changes in the optical signal amplitude distribution were noted to correspond to tissue thickness in a thin tissue sample. Tissue surface activation opposing stimulus was heterogeneous likely due to tissue geometry and decreased conduction through adipose and collagen regions in the human atria. Future work includes the imaging and modeling of larger regions in the human atria and comparison to experimental optical mapping results.

10053-80, Session 12

Using speckle to measure tissue dispersion in optical coherence tomography

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In Optical Coherence tomography (OCT), dispersion mismatches cause degradation of the image resolution. However, dispersion is specific to the material that is causing the effect and can therefore carry diagnostically useful information from tissue. In this summary, we propose a novel technique for estimating the dispersion in tissue which uses the image speckle to calculate the PSF degradation and is therefore applicable to any tissue and can be implemented in vivo and in situ. To obtain a practical speckle-PSF estimation technique, a Wiener-type minimization was used leading to a Wiener-type deconvolution algorithm to estimate the image PSF degradation from the speckle. The proposed method was verified ex vivo resulting in comparable values of the Group Velocity Dispersion (GVD) as obtained by a standard estimation technique described in the literature. The applicability to cancer diagnosis was evaluated on a small set of gastrointestinal normal and cancer OCT images. Using the statistics of the GVD estimate, the tissue classification resulted in 93% sensitivity and 73% specificity (84% correct classification rate). The success of these preliminary results indicates the potential of the proposed method which should be further investigated to elucidate its advantages and limitations.

10053-81, Session 12

Visualization and tissue classification of human breast cancer images using ultrahigh-resolution OCT

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We employed a home-built ultrahigh resolution (UHR) OCT system at 800nm to image human breast cancer sample ex vivo. The system has an axial resolution of 2.72μm and a lateral resolution of 5.52μm with an extended imaging range of 1.78mm. Over 900 UHR OCT volumes were generated on specimens from 23 breast cancer cases. With better spatial resolution, detailed structures in the breast tissue were better defined. Different types of breast cancer as well as healthy breast tissue can be well delineated from the UHR OCT images. To quantitatively evaluate the advantages of UHR OCT imaging of breast cancer, features derived from OCT intensity images were used as inputs to a machine learning model, the relevance vector machine. A trained machine learning model was employed to evaluate the performance of tissue classification based on UHR OCT images for differentiating tissue types in the breast samples, including adipose tissue, healthy stroma and cancerous region. For adipose tissue, grid-based local features were extracted from OCT intensity data, including standard deviation, entropy, and homogeneity. We showed that it was possible to enhance the classification performance on distinguishing fat tissue from non-fat tissue by using the UHR images when compared with the results based on OCT images from a commercial 1300 nm OCT system. For invasive ductal carcinoma (IDC) and normal stroma differentiation, the classification was based on frame-based features that portray signal penetration depth and tissue reflectivity. The confusing matrix indicated a sensitivity of 97.5% and a sensitivity of 77.8%.

10053-82, Session 12

Complex averaging in optical coherence tomography: a way to reduce the effect of multiple scattering and improve image contrast

Lars Thrane, Shi Gu, Brecken J. Blackburn, Kishore V. Damodaran, Andrew M. Rollins, Michael W. Jenkins, Case Western Reserve Univ. (United States)

The extensive development of frequency-domain optical coherence tomography (OCT) for more than a decade has enabled A-scan rates in the MHz range. Furthermore, frequency-domain OCT gives access to the amplitude and phase of the OCT signal. These characteristics have opened the possibilities of doing different kinds of averaging in order to improve OCT imaging. It is well known that multiple scattering in OCT reduces image contrast and resolution especially at greater depths within the tissue. Here, we demonstrate that complex averaging can decrease the effect of multiple

scattering and improve OCT imaging contrast, in addition to increasing the dynamic range due to reducing the noise floor as previously demonstrated. We take advantage of the fact that complex averaging, in contrast to conventional magnitude averaging, is sensitive to phase changes, as one averages the complex-valued Fourier-transformed spectral fringe signals before calculating the magnitude. Any motion that leads to higher phase variance will lead to lower magnitude when performing complex averaging. Also, motion preferentially increases the phase variance of multiply scattered photons when compared to singly scattered photons with each scattering event spreading the phase. This indicates that we may reduce multiple scattering by implementing complex averaging to preferentially reduce the magnitude of the multiply scattered light signal in OCT images. We have performed several experiments on liquid phantoms that give experimental evidence for this hypothesis.

10053-83, Session 12

A stochastically fully connected conditional random field framework for super resolution OCT

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A number of factors can degrade the resolution and contrast of OCT images, such as: (1) changes of the OCT point-spread function (PSF) resulting from wavelength dependent scattering and absorption of light along the imaging depth (2) speckle noise, as well as (3) motion artifacts. We propose a new Super Resolution OCT (SR OCT) imaging framework that takes advantage of a Stochastically Fully Connected Conditional Random Field (SF-CRF) model to generate a Super Resolved OCT (SR OCT) image of higher quality from a set of Low-Resolution OCT (LR OCT) images. The proposed SF-CRF SR OCT imaging is able to simultaneously compensate for all of the factors mentioned above, that degrade the OCT image quality, using a unified computational framework. The proposed SF-CRF SR OCT imaging framework was tested on a set of simulated LR human retinal OCT images generated from a high resolution, high contrast retinal image, and on a set of in-vivo, high resolution, high contrast rat retinal OCT images. The reconstructed SR OCT images show considerably higher spatial resolution, less speckle noise and higher contrast compared to other tested methods. Visual assessment of the results demonstrated the usefulness of the proposed approach in better preservation of fine details and structures of the imaged sample, retaining biological tissue boundaries while reducing speckle noise using a unified computational framework. Quantitative evaluation using both Contrast to Noise Ratio (CNR) and Edge Preservation (EP) parameter also showed superior performance of the proposed SF-CRF SR OCT approach compared to other image processing approaches.

10053-84, Session 12

High contrast and polarization-artifact-free optical coherence tomography by maximum a-posteriori estimation

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We propose maximum a-posteriori (MAP) intensity estimation to improve contrast and signal-to-noise ratio (SNR) and demonstrate its application to polarization diversity (PD)-OCT imaging to achieve high-contrast polarization-artifact-free images. PD detection inevitably reduces SNR due to the splitting of power to two polarization detection channels. To compensate we adopt maximum a-posteriori (MAP) estimation from four repeated B-scans, resulting in high contrast OCT images with polarization artifact suppression. To evaluate this image composition method, we make use of a 1-μm multifunctional Jones matrix OCT system to mimic non-PD and PD-OCT. Non-PD and PD-OCT images are each combined with the methods of intensity averaging or MAP estimation of four repeated B-scans. Images of the posterior eye were taken at the macula and ONH. Results show that the combination of MAP and PD-OCT is most optimal, as a high retinal pigment epithelium to vitreous image intensity ratio, or SNR, is obtained while birefringence artifacts in the peripapillary sclera region are suppressed. Non-PD-OCT images combined with MAP can be a good choice for imaging non-birefringent samples as it can generate the highest SNR images. Attenuation images generated from MAP estimated PD-OCT images show high-dynamic-range, polarization-artifact-free images with finer differentiation of attenuation levels.

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10054-1, Session 1

High wavenumber Raman spectroscopic characterization of normal and oral cancer using blood plasma

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Blood plasma possesses the biomolecules released from cells/tissues after metabolism and reflects the pathological conditions of the subjects. The analysis of biofluids for disease diagnosis becomes very attractive in the diagnosis of cancers due to the ease in the collection of samples, easy to transport, multiple sampling for regular screening of the disease and being less invasive to the patients. Hence, the intention of this study was to apply near-infrared (NIR) Raman spectroscopy in the high wavenumber (HW) region (2500?3400 cm?1) for the diagnosis of oral malignancy using blood plasma. From the Raman spectra it is observed that the biomolecules protein and lipid played a major role in the discrimination between groups. The diagnostic algorithms based on principal components analysis coupled with linear discriminant analysis (PCA-LDA) with the leave-one-patient-out cross-validation method on HW Raman spectra yielded a promising results in the identification of oral malignancy. The details of results will be discussed.

10054-2, Session 1

Tumor margin assessment in Mohs surgery using reflectance, fluorescence and Raman spectroscopy

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Mohs surgery is the current gold standard to treat large, aggressive or high-risk non-melanoma skin cancer (NMSC) cases.?While Mohs surgery is an effective treatment, the procedure is time-consuming and expensive for physicians as well as burdensome for patients as they wait for frozen section histology. Our group has recently demonstrated high diagnostic accuracy using a noninvasive “spectral biopsy” (combination of diffuse reflectance (DRS), fluorescence (FS) and Raman spectroscopy (RS)) to classify NMSC vs. normal lesion in a screening setting of intact tissue. Here, we examine the sensitivity of spectral biopsy to pathology in excised Mohs sections. The system is designed with three modalities integrated into one fiber probe, which is utilized to measure DRS, FS, and RS of freshly excised skin from patients with various NMSC pathologies including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), where each measurement location is correlated to histopathology. The spectral biopsy provides complimentary physiological information including the reduced scattering coefficient, hemoglobin content and oxygen saturation from DRS, NADH and collagen contribution from FS and information regarding multiple proteins and lipids from RS. We then apply logistic regression model to the extracted physiological parameters to classify NMSC vs. normal tissue. The results on the excised tissue are generally consistent with in vivo measurements showing decreased scattering within the tumor and reduced fluorescence. Due to the high sensitivity of RS to lipids, subcutaneous fat often dominates the RS signal. This pilot study demonstrates the potential for a spectral biopsy to classify NMSC vs. normal tissue, indicating the opportunity to guide Mohs excisions.

10054-3, Session 1

Raman spectroscopic characterization of urine of normal and cervical cancer subjects

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The metabolic waste products from cells are transferred to biofluids out of which few metabolites such as urea, creatinine and uric acid along with water are excreted through urine. For mass screening, urine is the best choice, due to its ease collection and less complex when compared to blood and tissues. In the present study, urine of both the normal and cancer patients were taken for Raman spectroscopy. Raman spectroscopy provides the specific band for the bio chemical products that were released by the metabolism. In this present work, Raman spectroscopy of urine of normal subjects and cervix cancer patients were studied with He-Ne laser of wavelength 633 nm. From the obtained spectra, the Raman bands at 1282 cm-1, 1351 cm-1 and 1468 cm-1 corresponds to amide III, renal cell and CH3 deformation due to peptide side chains played a major role in the classification between the normal and cancer groups. The spectral data of normal and cervical cancer subjects yielded a good accuracy when analyzed with multivariate statistical discriminant analysis. The results will be discussed in detail.

10054-4, Session 1

Rapid and accurate peripheral nerve detection using multipoint Raman imaging

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Nerve-sparing surgery is essential to avoid functional deficits of the limbs and organs. Raman scattering, a label-free, minimally invasive, and accurate modality, is one of the best candidate technologies to detect nerves for nerve-sparing surgery. However, Raman scattering imaging is too time-consuming to be employed in surgery. Here we present a rapid and accurate nerve visualization method using a multipoint Raman imaging technique that has enabled simultaneous spectra measurement from different locations (n=32) of a sample. Five sec is sufficient for measuring n=32 spectra with good S/N from a given tissue. Principal component regression discriminant analysis discriminated spectra obtained from peripheral nerves (n=863 from n=161 myelinated nerves) and connective tissue (n=828 from n=121 tendons) with sensitivity and specificity of 88.3% and 94.8%, respectively. To compensate the spatial information of a multipoint-Raman-derived tissue discrimination image that is too sparse to visualize nerve arrangement, we used morphological information obtained from a bright-field image. When merged with the sparse tissue discrimination image, a morphological image of a sample shows what portion of Raman measurement points in arbitrary structure is determined as nerve. Setting a nerve detection criterion on the portion of “nerve” points in the structure as 40% or more, myelinated nerves (n=161) and tendons (n=121) were

discriminated with sensitivity and specificity of 97.5%. The presented technique utilizing a sparse multipoint Raman image and a bright-field image has enabled rapid, safe, and accurate detection of peripheral nerves.

10054-5, Session 1

Label-free characterization of articular cartilage in osteoarthritis model mice by Raman spectroscopy

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Osteoarthritis (OA) is very common joint disease in the aging population. Main symptom of OA is accompanied by degenerative changes of articular cartilage. Cartilage contains mostly type II collagen and proteoglycans, so it is difficult to access the quality and morphology of cartilage tissue in situ by conventional diagnostic tools (X-ray, MRI, and echography) directly or indirectly. Raman spectroscopy is a label-free technique which enables to analyze molecular composition in degenerative cartilage. In this study, we generated an animal OA model surgically induced by knee joint instability, and the femurs were harvested at two weeks after the surgery. We performed Raman spectroscopic analysis for the articular cartilage of distal femurs in OA side and unaffected side in each mouse. In the result, there is no gross findings in the surface of the articular cartilage in OA. On the other hand, Raman spectral data of the articular cartilage showed drastic changes in comparison between OA and control side. The major finding of this study is that the relative intensity of phosphate band (960 cm-1) increases in the degenerative cartilage. This may be the result of exposure of subcondral bone due to thinning of the cartilage layer. In conclusion, Raman spectroscopic technique is sufficient to characterize articular cartilage in OA as a pilot study for Raman application in cartilage degeneration and regeneration using animal models and human subjects.

10054-6, Session 2

Feasibility of quantitatively diagnosing ocular infection using Raman spectroscopy

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Ocular infection is a serious eye disease that could lead to blindness without prompt and proper treatment. In pathology, ocular infection is caused by microorganisms such as bacteria, fungi or viruses. The essential prerequisite for the optimal treatment of ocular infection is to identify the microorganism causing infection early as each type of microorganism requires a different therapeutic approach. The clinical procedure for identifying the microorganism species causing ocular infection includes Gram staining (for bacteria)/microscopy (for fungi) and the culture of corneal surface scraping, or aqueous and vitreous smear samples taken from the surface of infected eyes. The culture procedure is labor intensive and expensive. Moreover, culturing is time consuming, which usually takes a few days or even weeks. Such a long delay in diagnosis could result in the exacerbation of patients' symptoms, the missing of the optimal time frame for initiating treatment and subsequently the rising cost for disease management. Raman spectroscopy has been shown highly effective for non-invasive identification of both fungi and bacteria qualitatively. In this study, we investigate the feasibility of identifying the microorganisms of ocular infection and quantifying the concentrations using Raman spectroscopy by measuring not only gram negative and gram positive bacteria but also infected cornea. By applying a modified orthogonal projection approach, the relative concentration of each bacteria species could be quantified. Our results indicate the great potential of Raman spectroscopy as an alternative tool for non-invasive diagnosis of ocular infection and could play a significantly role in future ophthalmology.

10054-7, Session 2

Channel-compressed spectrometry for multiplexed molecular imaging of biomarker-targeted SERS nanoparticles on fresh tissue specimens

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Biomarker-targeted surface-enhanced Raman scattering (SERS) nanoparticles (NPs) have been explored as a viable option for targeting and imaging multiple cell-surface protein biomarkers of cancer. While it has been demonstrated that this Raman-encoded molecular imaging (REMI) technology may potentially be used to guide tumor-resection procedures, the REMI strategy would benefit from further improvements in imaging speed. Previous implementations of REMI have utilized 1024 spectral channels (camera pixels) in a commercial spectroscopic CCD to detect the spectral signals from multiplexed SERS NPs, a strategy that enables accurate demultiplexing of the relative concentration of each NP "flavor" within a mixture. Here, we investigate the ability to significantly reduce the number of spectral-collection channels while maintaining accurate imaging and demultiplexing of up to five SERS NP flavors, a strategy that offers the potential for improved imaging speed and/or detection sensitivity in future systems. This strategy was optimized by analyzing the linearity of five multiplexed flavors of SERS NPs topically applied on tissues. The accuracy of this binning approach was then validated by staining tumor xenografts and human breast tumor specimens with a mixture of five NP flavors (four targeted NPs and one untargeted NP) and performing ratiometric imaging of specific vs. nonspecific NP accumulation. We demonstrate that with channel-compressed spectrometry using as few as 16 channels, it is possible to perform REMI with five NP flavors, with <20% error, at low concentrations (<10 pM) that are relevant for clinical applications.

10054-8, Session 2

In-situ shifted excitation Raman difference spectroscopy: development and demonstration of a portable sensor system at 785 nm

Martin Maiwald, André Müller, Bernd Sumpf, Ferdinand-Braun-Institut (Germany)

Portable Raman spectroscopy is becoming increasingly important for various application fields such as point-of-care diagnostic. Real-world measurements are often disturbed by fluorescence and ambient light. Shifted excitation Raman difference spectroscopy (SERDS) is demonstrated as a powerful and easy-to-use technique to separate the Raman signals from disturbing background signals. Moreover, for many in-situ applications, such as in-vivo diagnostics, a fast visualization and evaluation of undisturbed Raman signals are needed for rapid on-site decisions.

In this contribution, a novel portable SERDS sensor system will be presented. The device includes an in-house developed handheld Raman probe with an implemented dual wavelength diode laser at 785 nm. First, the portable SERDS sensor system will be described. After the description, the performance of the instrument will be discussed. Here, an excitation power of 120 mW is achieved ex probe for both emission wavelengths with a spectral distance of 10 cm-1 for SERDS. Raman experiments in the laboratory using polystyrene as test sample will demonstrate the suitability of the device.

Finally, the above described portable SERDS system was applied in an apple orchard for demonstration and these outdoor experiments will be presented. Apples and apple leafs were excited with 50 mW optical power and were used as test samples. For green leafs the exposure time was reduced to 0.2 s to avoid detector saturation. SERDS efficiently extracts the Raman signals and generates an 11-fold improvement of the signal-to-background noise. The results demonstrate the capability of the portable SERDS system and enable rapid in situ and undisturbed Raman investigations.

10054-9, Session 2

A stepwise spectral reconstruction method for spectroscopic Raman imaging

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Raman spectroscopy has demonstrated great potential in biomedical applications. However, spectroscopic Raman imaging is not widely used because of slow data acquisition. Our previous studies have indicated that spectroscopic Raman imaging can be significantly sped up using the approach of narrow-band imaging followed by spectral reconstruction. A multi-channel system has been built to demonstrate the feasibility of fast wide-field Raman spectroscopic imaging based on simultaneous narrow-band image acquisition and spectral reconstruction based on Wiener estimation in phantoms. To further improve the accuracy of reconstructed Raman spectra, we propose a stepwise spectral reconstruction method in this study, which can be combined with the earlier developed sequential weighted Wiener estimation to improve the spectral reconstruction accuracy. The stepwise spectral reconstruction method first reconstructs the fluorescence background spectra from narrow-band measurements by sequential weighted Wiener estimation and then the pure Raman narrow-band measurements can be estimated by subtracting the estimated fluorescence background from the overall Raman measurements. Thereafter, pure Raman spectra can be reconstructed from estimated pure Raman narrow-band measurements. The result indicates that the stepwise spectral reconstruction method can improve the spectral reconstruction accuracy by more than 30% when combined with sequential weighted Wiener estimation, compared with traditional Wiener estimation. In addition, cell Raman imaging were realized by using a multi-channel wide field Raman spectroscopic imaging and the stepwise spectral reconstruction method. This method can potentially facilitate the use of spectroscopic Raman imaging to investigate fast changing phenomena in biological samples.

10054-10, Session 2

Parameter optimization in Raman spectroscopy for differentiation of neural progenitor cells and their lineages

Keren Chen, William Ong, Sing Yian Chew, Quan Liu, Nanyang Technological Univ. (Singapore)

Neurological diseases are one of the leading causes of adult disability and they are estimated to cause more deaths than cancer in the elderly population by 2040. Stem cell therapy has shown great potential in treating neurological diseases. However, before cell therapy can be widely adopted in the long term, a number of challenges need to be addressed, including the fundamental research about cellular development of neural progenitor cells. To facilitate the fundamental research of neural progenitor cells, many methods have been developed to identify neural progenitor cells. Although great progress has been made, there is still lack of an effective method to achieve fast, label-free and noninvasive differentiation of neural progenitor cells and their lineages. As a fast, label-free and noninvasive technique, spontaneous Raman spectroscopy has been conducted to characterize many types of stem cells including neural stem cells. However, to our best knowledge, it has not been studied for the discrimination of neural progenitor cells from specific lineages. Here we report the differentiation of neural progenitor cell from their lineages including astrocytes, oligodendrocytes and neurons using spontaneous Raman spectroscopy. Moreover, we also evaluate the influence of system parameters during spectral acquisition on the quality of measured Raman spectra and the accuracy of classification using the spectra, which yield a set of optimal system parameters facilitating future studies.

10054-51, Session 2

DNA detection and single nucleotide mutation identification using SERS for molecular diagnostics and global health

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Nucleic acid-based molecular diagnostics at the point-of-care (POC) and in resource-limited settings is still a challenge due to the lack of sensitive and practical DNA detection methods that can be seamlessly integrated into portable platforms. We present a sensitive yet simple DNA detection method with single nucleotide polymorphism (SNP) discrimination capability. Surface-enhanced Raman scattering (SERS) was used as the readout method. The detection scheme involves sandwich hybridization of magnetic beads that are loaded with capture probes, target sequences, and ultrabright SERS nanorattles with reporter probes. Upon hybridization, the sandwich probes are concentrated at the detection focus controlled by a magnetic system for SERS measurements. The ultrabright SERS nanorattles, consisting of a core and a shell with resonance Raman reporters loaded in the gap space, serve as SERS tags for ultrasensitive signal detection. A specific DNA sequence of the malaria parasite Plasmodium falciparum was used as the model marker system. Detection limit of approximately 100 attomoles was achieved. Single nucleotide polymorphism (SNP) discrimination of wild type malaria DNA and mutant malaria DNA, which confers resistance to artemisinin drugs, was also demonstrated. The results demonstrate the molecular diagnostic potential of the nanorattle-based method to both detect and genotype infectious pathogens. The method's simplicity makes it a suitable candidate for molecular diagnostics at the POC and in resource-limited settings.

10054-11, Session 4

Hyperspectral imaging of colonic polyps in vivo

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Standard endoscopic tools restrict clinicians to making subjective visual assessments of lesions detected in the bowel, with classification results depending strongly on experience level and training. Histological examination of resected tissue remains the diagnostic gold standard, meaning that all detected lesions are routinely removed. This subjects the patient to risk of polypectomy-related injury, and places significant workload and economic burdens on the hospital. An objective endoscopic classification method would allow hyperplastic polyps, with no malignant potential, to be left in situ, or low grade adenomas to be resected and discarded without histology. A miniature multimodal flexible endoscope is proposed to obtain hyperspectral reflectance and dual excitation autofluorescence information from polyps in vivo. This is placed inside

the working channel of a conventional colonoscope, with the external scanning and detection optics on a bedside trolley. A blue and violet laser diode pair excite endogenous fluorophores in the respiration chain, while the colonoscope's xenon light source provides broadband white light for diffuse reflectance measurements. A push-broom HSI scanner collects the hypercube. System characterisation experiments are presented, defining resolution limits as well as acquisition settings for optimal spectral, spatial and temporal performance. The first in vivo results in human subjects are presented, demonstrating the clinical utility of the device. The optical properties (reflectance and autofluorescence) of imaged polyps are quantified and compared to the histologically-confirmed tissue type as well as the clinician's visual assessment. Further clinical studies will allow construction of a full robust training dataset for development of classification schemes.

10054-12, Session 4

Tumor margin assessment of surgical tissue specimen using hyperspectral imaging and machine learning

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Of the 15.2 million new cases of cancer each year, over 80% of cases will need surgery. The single most important predictor of patient survival for almost all solid cancers is a complete surgical resection. We are developing a label-free hyperspectral imaging (HSI) and machine learning approach for objective assessment of cancer margins. HSI data, hypercube (x,y,?), consists of a series of high-resolution (~20 μm) images of the same field of view that are acquired at different wavelengths (450-950 nm at 2 nm interval). Every pixel in the HSI image has an optical spectrum. We developed a pipeline of processing and quantification tools for HSI data, which include spectral normalization, image registration, glare detection, and curvature correction. We also developed feature extraction methods to extract image and spectral features from HSI data for the classification of cancer and benign tissue. We compared 11 different classification algorithms and tested our HSI approach in eight tumor-bearing mice. We collected surgical tissue specimens from 28 patients who underwent head and neck (H&N) cancer surgery. We acquired both HSI and fluorescence images (2-NBDG and proflavine) from the specimens. Digitized histologic slides were examined by an H&N pathologist. Our human study showed that label-free HSI, proflavine, and 2-NBDG have an accuracy of 79.5%, 85.5%, and 90.3% for H&N cancer detection, respectively. We demonstrated the feasibility of using HSI for surgical margin assessment in animals. Further development and testing of the HSI technology is needed for its use in human surgery.

10054-13, Session 4

Three-dimensional online surface reconstruction of augmented fluorescence lifetime maps using photometric stereo

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Multi-Spectral Time-Resolved Fluorescence Spectroscopy (ms-TRFS) can provide label-free real-time feedback on tissue composition and pathology during surgical procedures by resolving the fluorescence decay dynamics of the tissue. Recently, an ms-TRFS system has been developed in our group, allowing for either point-spectroscopy fluorescence lifetime measurements or dynamic raster tissue scanning by merging a 450 nm aiming beam with the pulsed fluorescence excitation light in a single fiber collection. In order to facilitate an augmented real-time display of fluorescence decay parameters, the lifetime values are back projected to the white light video. The goal of this study is to develop a 3D real-time surface reconstruction aiming for a comprehensive visualization of the decay parameters and providing an enhanced navigation for the surgeon. Using a stereo camera setup, we use a combination of image feature matching and aiming beam stereo segmentation to establish a 3D surface model of the decay parameters. After camera calibration, texture-related features are extracted for both camera images and matched providing a rough estimation of the surface. During the raster scanning, the rough estimation is successively refined in real-time by tracking the aiming beam positions using an advanced segmentation algorithm. The method is evaluated for excised breast tissue specimens showing a high precision and running in real-time with approximately 20 frames per second. The proposed method shows promising potential for intraoperative navigation, i.e. tumor margin assessment. Furthermore, it provides the basis for registering the fluorescence lifetime maps to the tissue surface adapting it to possible tissue deformations.

10054-14, Session 4

Multiphoton fluorescence microscopy using a 1μm fiber laser for rapid evaluation of breast surgical specimens

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Intra-surgical evaluation of breast surgical specimens during breast conserving surgery, a procedure that has a repeat surgery rate of up to 40%, has significant potential to reduce the rates of repeat surgeries due to positive or close margins. The multi-hour processing time required for standard paraffin embedded hematoxylin and eosin histopathology makes it preclusive for intra-surgical use. Frozen section analysis (FSA) can be performed more rapidly, but has limited sensitivity and sectioning of fatty tissue is difficult. Multiphoton microscopy's ability to provide axially sectioned, high resolution images of fluorescently stained tissue, makes it desirable for intra-surgical tissue evaluation. Our group previously reported high sensitivities and specificities for evaluating breast cancer pathology versus normal or benign pathology on multiphoton microscopy images acquired with a bench top microscope using a Ti:Sapphire laser [1]. For translation of this technology to the clinic, a more compact and lower cost microscope is advantageous.

In this work, we describe a technique for rapid evaluation of unfixed breast tissue specimens using multiphoton microscopy with a 1μm fiber laser. A novel staining procedure enables us to rapidly stain and fluorescently excite, at 1μm wavelength, nuclear and stromal components of tissue and generate images analogously to H&E. The longer wavelength light enables deeper imaging when compared to more common, shorter wavelength excitation with Ti:Sapphire lasers at 780nm. The all-fiber laser does not require bulky power supplies, chillers, or controllers, significantly reducing the footprint and cost of the system. We present a comparative study imaging freshly excised breast tissue pathology using virtual H&E color-remapped multiphoton microscope images and standard H&E.

[1] Y. K. Tao, et al., "Assessment of breast pathologies using nonlinear microscopy," Proc. Natl. Acad. Sci., 2014.

10054-15, Session 4

Detection of radiation-induced brain necrosis in live rats using label-free time-resolved fluorescence spectroscopy

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Differentiating radiation-induced necrosis from recurrent tumor in the brain remains a significant challenge to the neurosurgeon. Clinical imaging modalities are not able to reliably discriminate the two tissue types, making biopsy location selection and surgical management difficult. Label-free fluorescence lifetime techniques have previously been shown to be able to delineate human brain tumor from healthy tissues. Thus, fluorescence lifetime techniques represent a potential means to discriminate the two tissues in real-time during surgery. This study aims to characterize the endogenous fluorescence lifetime signatures from radiation induced brain necrosis in a tumor-free rat model. Fischer rats received a single fraction of 60 Gy of radiation to the right hemisphere using a linear accelerator. Animals underwent a terminal live surgery after gross necrosis had developed, as verified with MRI. During surgery, healthy and necrotic brain tissue was measured with a fiber optic needle connected to a multispectral fluorescence lifetime system. Measurements of the necrotic tissue showed a 48% decrease in intensity and 20% increase in lifetimes relative to healthy tissue. Using a support vector machine classifier and leave-one-out validation technique, the necrotic tissue was correctly classified with 94% sensitivity and 97% specificity. Spectral contribution analysis also confirmed that the primary source of fluorescence contrast lies within the redox and bound-unbound population shifts of nicotinamide adenine dinucleotide. A clinical trial is presently underway to measure these tissue types in humans. These results show for the first time that radiation-induced necrotic tissue in the brain contains significantly different metabolic signatures that are detectable with label-free fluorescence lifetime techniques.

10054-16, Session 4

Detection of human brain tumor infiltration with multimodal multiscale optical analysis

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Brain tumor surgeries are facing major challenges to improve patients' quality of life. The extent of resection, measured by Simpson grading system, while preserving surrounding eloquent brain areas is necessary to equilibrate the onco-functional. A tool able to increase the accuracy of tissue analysis and that will be able on long term to deliver an immediate diagnostic on tumor, could drastically improve actual surgeries and patients survival rates. To achieve such performances a complete optical study, ranging from ultraviolet to infrared, of biopsies has been started by our group. Four different contrasts were used by our team: 1) spectral analysis covering the DUV to IR range, 2) two photon fluorescence lifetime imaging and one photon time domain measurement, 3) second harmonic generation imaging and 4) Fluorescence imaging using DUV to IR, by one and two photon excitation. All those measures were recorded from the endogenous fluorescence of tissues to avoid any bias and further clinical complication due to exogenous markers; thereafter, all the different modalities are crossed to build a matrix of criteria to discriminate tumorous tissues. The resulting score of multimodal optical analysis on human biopsies were compared to the gold standard histopathology.

10054-45, Session PSun

Research of venous and capillary blood analytes using Raman spectroscopy approach

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Changes in the biofluids (blood plasma, whole blood, protein fractions) concentration is among the most important criteria for early diagnosis and assessment of the oncohematological diseases progression. There is a major interest in optical measurement that would permit simultaneous analysis of multiple components in whole blood without the need for conventional sample processing, such as centrifuging and reagents adding. The major challenge of whole blood samples analysis is a presence of numerous low-concentration components. This work is devoted to study the possibility of plasma proteins concentration measurement using Raman spectroscopy (RS) setup. This work is devoted to study venous and capillary blood analytes using Raman spectroscopy setup. The blood proteins and whole blood were examined in this research.

Raman spectra were measured by integrated Raman setup. The setup comprises of thermally stabilized diode laser LML-785.0RB-04 (785 nm, 200 mW), low-cost commercial Raman probe and spectrograph Shamrock SR-500i-D1-R with deep cooling digital camera Andor iDus DU416A-LDC-DD up to -70ºC. The acquisition time was 60 seconds for signal registration from biofluids. Biofluid samples were located in the aluminium cuvette with different reflector shape. Pure Raman spectrum was achieved by autofluorescence removal from raw data signal using polynomial approximation method.

Current research was associated with the determination of abnormal protein concentrations values in a whole blood and blood plasma by Raman spectroscopy approach. For data processing PLS regression was utilized. The root mean squared error (RMSE) and squared correlation coefficient (R2) were calculated for results estimation.

10054-46, Session PSun

Evaluation and diagnosis of brain death by Functional near-infrared spectroscopy

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Brain death, the irreversible and permanent loss of the brain and brainstem functions, is hard to be judged precisely for some clinical reasons. The traditional diagnostic methods are time consuming, expensive and some are even dangerous. Functional near infrared spectroscopy (fNIRS), using the good scattering properties of major component of blood to NIR, is capable of noninvasive monitoring cerebral hemodynamic responses. Here, we attempt to use portable fNIRS under patients' natural state for brain death diagnosis. Ten brain death patients and seven normal subjects participated

in fNIRS measurements. All of them were provided different fractional concentration of inspired oxygen (FIO2) in different time periods. We found that the concentration variation of deoxyhemoglobin concentration (?[Hb]) in brain death patients is significantly lower than normal subjects, and emerges the rising trend as time went on. With the raise of the FIO2, ?[Hb] presents the trend of decrease in the both brain death patients and normal subjects, however, the data in the brain death patients is more significant. And the concentration variation of oxyhemoglobins concentration emerges the opposite trends. The findings indicated the potential of fNIRS-measured hemodynamic index in diagnosing brain death.

10054-47, Session PSun

Detection of migraine by using near-infrared spectroscopy

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Migraine is considered as a neurovascular coupling disorder due to the impaired cerebrovascular reactivity which is characterized by recurrent moderate to severe headaches. Migraine usually leads the patients to disability. Migraine have multiple types, but we will focus on episodic migraine (EM) and chronic migraine (CM) in this study.

The present study uses functional near-infrared spectroscopy (fNIRS) system to detect the changes of oxyhemoglobin (HbO2) and deoxyhemoglobin (Hb) in prefrontal cortex of healthy people and patients with migraine under breath holding test. Discuss the relation between the changes of hemoglobin in prefrontal cortex and neurological disorder characterized by headaches.

10054-48, Session PSun

Localization of subsurface photoacoustic fiducials for intraoperative guidance

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Intraoperative guidance is often used during procedures to provide surgeons with information linking the current surgical scene to some preoperative plan or model. This allows surgeons to visualize structures or targets of interest that are not visible in the intraoperative imaging modality. A common method to enable this type of technology is the use of fiducials that can be located both preoperatively and intraoperatively. In this work, we focus on the registration of preoperative computed tomography with intraoperative three-dimensional ultrasound. We examine the use of titanium fiducials activated intraoperatively by the photoacoustic effect. The photoacoustic effect is generated when the metal fiducials are illuminated by laser light, resulting in an acoustic signal at each fiducial that can be observed by a conventional ultrasound transducer. A transrectal ultrasound transducer is translated to generate a three-dimensional ultrasound volume. The set of fiducial points are segmented from the three-dimensional ultrasound volume and registered with the corresponding set of fiducial points segmented from the computed tomography volume. The target registration error metric is used to validate the registration between these two coordinate systems. The resulting target registration error was 1.55 ± 0.62mm. It was observed in this experiment that there were certain acoustic reverberation and refraction artifacts that occurred at distances coinciding with the size of the fiducial. Further work with different shapes and sizes of fiducials may be necessary to quantify and analyze how they affect the generation of a photoacoustic signal.

10054-49, Session PSun

Raman spectroscopic detection of peripheral nerves towards nerve-sparing surgery

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The peripheral nervous system plays an important role in motility, sensory, and autonomic functions of the human body. Preservation of peripheral nerves in surgery, namely nerve-sparing surgery, is now promising technique to avoid functional deficits of the limbs and organs following surgery as an aspect of the improvement of quality of life of patients. Detection of peripheral nerves including myelinated and unmyelinated nerves is required for the nerve-sparing surgery; however, conventional nerve identification scheme is sometimes difficult to identify peripheral nerves due to similarity of shape and color to non-nerve tissues or its limited application to only motor peripheral nerves. To overcome these issues, we proposed a label-free detection technique of peripheral nerves by means of Raman spectroscopy. We found several fingerprints of peripheral myelinated and unmyelinated nerves by employing a modified principal component analysis of typical spectra including myelinated nerve, unmyelinated nerve, and adjacent tissues. We finally realized the sensitivity of 94.2% and the selectivity of 92.0% for peripheral nerves including myelinated and unmyelinated nerves against adjacent tissues. Although further development of an intraoperative Raman spectroscopy system is required for clinical use, our proposed approach will serve as a unique and powerful tool for peripheral nerve detection for nerve-sparing surgery in the future.

10054-50, Session PSun

Polarized Raman spectroscopic characterization of normal and oral cancer blood plasma

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In India oral cancer ranks the top due to the habitual usage of tobacco in its various forms and remains the major burden. Hence priority is given for early diagnosis as it is the better solution for cure or to improve the survival rate. For the past three decades, optical spectroscopic techniques have shown its capacity in the discrimination of normal and malignant samples. Many research works have conventional Raman in the effective detection of cancer using the variations in bond vibrations of the molecules. However in addition polarized Raman provides the orientation and symmetry of biomolecules. If so can polarized Raman be the better choice than the conventional Raman in the detection of cancer? The present study aimed to found the answer for the above query. The conventional and polarized Raman spectra were acquired for the same set of blood plasma samples of normal subjects and oral malignant (OSCC) patients. Thus, obtained Raman spectral data were compared using linear discriminant analysis coupled with artificial neural network (LDA-ANN). The depolarization ratio of biomolecules such as antioxidant, amino acid, protein and nucleic acid bases present in blood plasma was proven to be the best attributes in the categorization of the groups. The polarized Raman results were promising in discriminating oral cancer blood plasma from that of normal blood plasma with improved efficiency. The results will be discussed in detail.

10054-17, Session 5

Optical coherence tomography guided laser ablation endoscopic imaging system for minimal invasive laryngeal surgery

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Laser ablation surgery has been widely used in clinic for over decades. Especially for laryngeal surgeries, doctors choose laser ablation surgery in order to avoid large damage of tissue, such as vocal cord. And those kind surgeries usually will guide by laryngoscope which will only provide superficial image of the surgical tissue, the depth information will not be reviewed. Without the depth view of ablation tissue, it would be challenge to avoid the heath tissue and to eliminate the diseased tissue. In this study, a combine 1.3?m swept source optical coherence tomography (OCT) system with the laryngoscope are designed and fabricated. The combined endoscope could provide high resolution depth images and the superficial white light images simultaneously. The OCT and white light imaging region are designed to be co-registered with the laser ablation beam focus area, in order to guarantee the efficiency of the surgery. Ex vivo studies show that the combined OCT and laryngoscope could provide high resolution depth structure and the superficial structure of the tissue sample. And the results also demonstrate the imaging system are capable of co-registration with the laser ablation beam in laser ablation surgery process.

10054-18, Session 5

OCT imaging needles for deep anterior lamellar keratoplasty

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We developed a useful image-guided surgical tool of the fluid-injectable syringe needle that can obtain the OCT images with a very thin OCT probing optics. All the optical elements are equipped inside a thin syringe needle (standard 26G) in a robust form. The needle does not need an additional window that may weaken the mechanical properties. Instead, the OCT imaging is performed through the needle's open bevel in our OCT imaging needle. We applied our tool in the ophthalmic procedures of deep anterior lamellar keratoplasty (DALK). In the DALK procedures of corneal transplant, a syringe needle has been used to detach the Descemet's membrane by injecting the air in the lower layers of the stroma and forming a 'Big Bubble' inside the cornea. Here, the depth of air injection is a very important parameter for successful DALK, which has, however, depended only on the surgeon's experience in the current surgical techniques. We tested our imaging needle in the big bubble formation with human corneas, ex vivo, and rabbits', in vivo, to find the feasibility for DALK. We found that the OCT images obtained by the needle could provide the exact depth information on where the needle's injection point was while the needle's penetrating in the cornea. The image-guided surgery was found to make the DALK procedures more successful and reliable. We believe that our imaging needle can be a useful surgical tool not only for the ophthalmic applications but for other procedures that require precision injection of the air or liquids.

10054-19, Session 5

Spectroscopic optical coherence tomography for ex vivo brain tumor analysis

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For neurosurgeries precise tumor resection is essential for the subsequent recovery of the patients since nearby healthy tissue that may be harmed has a huge impact on the life quality after the surgery. However, so far no satisfying methodology has been established to assist the surgeon during surgery to distinguish between healthy and tumor tissue. Optical Coherence Tomography (OCT) potentially enables non-contact in vivo image acquisition at penetration depths of 1-2 mm with a resolution of approximately 1-15 μm. To analyze the potential of OCT for distinction between brain tumors and healthy tissue, we used a commercially available Thorlabs Callisto system to measure healthy tissue and meningioma samples ex vivo. All samples were measured with the OCT system and three dimensional datasets were generated. Afterwards they were sent to the pathology for staining with hematoxylin and eosin and then investigated with a bright field microscope to verify the tissue type. This is the actual gold standard for ex vivo analysis. The images taken by the OCT system exhibit variations in the structure for different tissue types, but these variations may not be objectively evaluated from raw OCT images. Since an automated distinction between tumor and healthy tissue would be highly desirable to guide the surgeon, we applied Spectroscopic Optical Coherence Tomography to further enhance the differences between the tissue types. Pattern recognition and machine learning algorithms were applied to classify the derived spectroscopic information. Finally, the classification results are analyzed in comparison to the histological analysis of the samples.

10054-20, Session 5

Optical coherence tomography guided smart laser knife for cancer surgery

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Neurological cancer surgeries require specialized tools that enhance imaging for precise cutting and removal of tissue without damaging adjacent neurological structures. The novel combination of high-resolution fast optical coherence tomography (OCT) alongside short pulsed nanosecond thulium (Tm) lasers offers stark advantages utilizing the superior beam quality, high volumetric tissue removal rates of thulium lasers with minimal residual thermal footprint in the tissue and avoiding damage to delicate sub-surface structures (e.g., nerves and microvessels); which has not been showcased before. A bench-top system is constructed, using a 15W 1940nm nanosecond pulsed Tm fiber laser (500uJ pulse energy, 100ns pulse duration, 30kHz repetition rate) for removing tissue and a swept source laser (1310±70nm, 100kHz sweep rate) is utilized for OCT imaging, forming a combined Tm/OCT system – a smart laser knife. The OCT image-guidance informs the Tm laser for cutting/removal of targeted tissue structures.

Tissue phantoms were constructed to demonstrate surgical incision with blood vessel avoidance on the surface where 2mm wide 600um deep cuts are executed around the vessel using OCT to guide the procedure. Cutting up to delicate subsurface blood vessels (2mm deep) is demonstrated while avoiding damage to their walls. A tissue removal rate of 5mm^3/sec is obtained from the bench-top system. We constructed a blow-off model to characterize Tm cut depths taking into account the absorption coefficients and beam delivery systems to compute Arrhenius damage integrals. The model is used to compare predicted tissue removal rate and residual thermal injury with experimental values in response to Tm laser-tissue modification.

10054-21, Session 6

Pancreatic cancer diagnosis based on full field OCT and dynamic full field OCT

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Pancreatic cancer is one of the most feared cancer types due to high death rates and the difficulty to perform surgery. This cancer outcome could benefit from recent technological developments for diagnosis. We used a combination of standard Full Field OCT and Dynamic Full Field OCT to capture both morphological features and metabolic functions of rodents pancreas in normal and cancerous conditions with and without chemotherapy. Results were compared to histology to evaluate the performances and the specificities of the method. The comparison highlighted the importance of a number of endogenous markers like immune cells, fibrous development, architecture and more.

10054-22, Session 6

Multimodal texture analysis of OCT images as a diagnostic application for skin tumors

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Optical coherence tomography (OCT) is an effective tool for determination of pathological topology that reflects structural and textural metamorphoses of tissue. In this paper, we propose a report about our examining of the validity of OCT in identifying changes using a skin cancer texture analysis compiled from Haralick texture features, fractal dimension, the complex directional field features and the Markov random field method from different tissues. Speckle reduction is an essential pre-processing part for OCT image analyze. In this work, we used an interval type-II fuzzy anisotropic diffusion algorithm for speckle noise reduction in OCT images (B- and/or C-scans). The Haralick texture features as contrast, correlation, energy, and homogeneity have been calculated in various directions. A box-counting method is performed to evaluate fractal dimension of skin probes. The complex directional field calculated by the local gradient methodology provides important data for linear dividing of species. We also estimate autocorrelation function using Markov random fields. Finally, the Boosting has been used to combine all heterogeneous texture method introduced before to single multimodal texture analysis method for the quality enhancing of the diagnostic method. Our results demonstrate that these texture features may present helpful information to discriminate tumor from healthy tissue. The experimental data set contains 400 OCT images with normal skin and tumors as Basal Cell Carcinoma (BCC), Malignant Melanoma (MM) and Nevus. We obtained sensitivity about 96% and specificity about 99% for a task of discrimination between MM and Nevus.

10054-23, Session 6

Diagnosis potential of hybrid Raman spectroscopy and optical coherence tomography technique for oral malignant lesions

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In this work, hybrid Raman spectroscopy (RS) and optical coherence tomography (OCT) technique was employed for the oral malignant lesions diagnosis. A side-view handheld RS-OCT optical probe is designed to co-align the optical paths of RS and OCT sampling arms. While RS reveals Raman spectral differences between normal and malignant oral tissues that can be attributed to the differences in inter- and intra-cellular proteins, lipids, DNA and water structures and conformations, enlightening biochemical changes associated with oral malignancy development; Simultaneously, OCT casts light on the tissue morphology changes accompanying the oral malignancy.

10054-24, Session 6

Towards double-clad fiber-based endoscopic single-pulse coagulation and concurrent optical coherence tomography

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There is a strong clinical need for an optical coherence tomography (OCT) system capable of concurrently delivering coagulation light to enable image-guided dynamic laser marking for targeted collection of biopsies and reduce false-negative findings common of conventional random sampling. Here, we present a system based on double-clad fiber (DCF) capable of delivering pulsed laser light through the inner cladding while performing OCT through the core. A clinically validated commercial OCT system (NVisionVLE, Ninepoint Medical) was adapted to accommodate DCF catheters by adding a DCF coupler and DCF-based rotary joint. The DCF coupler enabled combining both modalities into the DCF (core signal transmission: ~95%; inner cladding coupling: >80%). A DCF-based rotary joint (Princetel Inc.) was used to couple both the imaging and marking light to a spinning DCF-based catheter and perform helical scanning. In order to design catheters allowing optimal OCT imaging parameters along with a multimode beam spot size enabling single-pulse laser marking, Zemax modeling of the DCF was performed. In addition to the optical modeling, a photo-thermal model was used to identify the conditions enabling single-pulse laser marking. Catheter-dependent (beam spot size), laser source-dependent (power, pulse width, repetition rate, wavelength) and system-dependent (scanning speed, total transmission) parameters were included in the model. Combining the optical modeling and the photo-thermal model enables the investigation of different configurations of high-power laser diodes and catheter designs. Informed by this multi-parameter optimization, we are planning dynamic laser marking in swine to assess the capability of the system to achieve single-pulse coagulation in vivo.

10054-26, Session 7

Thrombolytic therapeutic effect monitoring based on functional near-infrared spectroscopy

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Deep vein thrombosis (DVT) can result in serious mortality and morbidity. The golden standard to diagnose DVT is venography, however, it relies on complicated imaging modalities that needed to be injected in a vein invasively. Noninvasive near-infrared spectroscopy (NIRS) has been reported recently that an intriguing and potential method detecting DVT in clinics. Arteriosclerosis obliterans (ASO), local extremities manifestations of systemic atherosclerosis, can result in thrombosis and the reduction of distal blood flow. Thrombolytic therapy uses exogenous activator to activate the dissolution system, which can dissolve intracoronary thrombus. Here we attempt to monitor the DVT and ASO patients during the whole procedure of thrombolytic treatment, then compare the data with those patients did not take treatments and normal population. 8 DVT and 9 ASO patients and 12 normal subjects were recruited to take the measurements of concentration variation of oxy- and deoxy-hemoglobins (?[HbO2] and ?[Hb]) by fNIRS-based thrombosis monitor. Thereinto, 5 DVT and 6 ASO patients has taken the thrombolytic treatment, and the data for the periods before treatment, during treatment, and after treatment were extracted for analysis. We found that ?[HbO2] fluctuates and even decreases in DVT and ASO patients. After the thrombolytic therapy, ?[HbO2] increases about 45% and converges to the curves of normal subjects. Whereas the ?[Hb] emerges the similar trends, except for the rising trend in the beginning and the downtrend after thrombolytic therapy. The findings indicate that NIRS has big potential in clinical monitoring of DVT and ASO patients and offering reliable and quantitative evaluation of thrombolytic therapy outcomes.

10054-27, Session 7

Development of a NIRS method to quantify cerebral perfusion and oxidative metabolism in preterm neonates with post hemorrhagic ventricle dilation

Peter McLachlan, Western Univ. (Canada); Jessica Kishimoto, Robarts Research Institute (Canada); Sandrine de Ribeaupierre, David S. C. Lee, Mamadou Diop, Keith St. Lawrence, Western Univ. (Canada)

A complication of intraventricular hemorrhage among preterm neonates is post-hemorrhagic ventricle dilation (PHVD), which is associated with a greater risk of life-long neurological disability. Clinical evidence, including suppressed EEG patterns, suggests that cerebral perfusion and oxygenation is impaired in these patients, likely due to elevated intracranial pressure (ICP). Cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen (CMRO2) can be quantified by dynamic contrast-enhanced NIRS; however, PHVD poses a unique challenge to NIRS since the cerebral mantle can be compressed to 1 cm or less. The objectives of this work were to develop a finite-slab model for the analysis of NIRS spectra, incorporating depth measurements from ultrasound images, and to assess the magnitude of error when using the standard semi-infinite model. CBF, tissue saturation (StO2) and CMRO2 were measured in 9 patients receiving ventricle taps to reduce ICP. Monte Carlo simulations indicated that errors in StO2 could be greater than 20% if the cerebral mantle was reduced to 1 cm. Using the finite-slab model, basal CBF and CMRO2 in the PHVD patients were not significantly different from a control group of preterm infants (14.6 ± 4.2 ml/100 g/min and 1.0 ± 0.4 ml O2/100 g/min), but StO2 was significantly lower (PDA 70.5 ± 9%, PHVD 58.9 ± 12%). Additionally, ventricle tapping improved CBF by 15.6 ± 22%. This work indicates that applying NIRS to PHVD patients is prone to error; however, this issue can be overcome with the appropriate model and using readily available ultrasound images.

10054-28, Session 7

Development of a hybrid broadband NIRS/diffusion correlation spectroscopy system for real-time monitoring of cerebral perfusion and oxygenation in preterm brain injury

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In Canada, 8% of births occur prematurely. Preterm infants weighing less than 1500g are at a high risk of neurodevelopmental impairment: 5-10% develop major disabilities such as cerebral palsy and 40-50% show other cognitive and behavioural deficits. The brain is vulnerable to periods of low cerebral blood flow (CBF) that can impair energy metabolism and cause tissue damage. There is, therefore, a need for an efficient neuromonitoring system to alert the neonatal intensive care team to clinically significant changes in CBF and metabolism, before injury occurs.

Optical technologies offer safe, non-invasive, and cost-effective methods for neuromonitoring. Cerebral oxygen saturation (ScO2) can be measured by exploiting the absorption properties of hemoglobin though Near-Infrared Spectroscopy (NIRS), and Diffuse Correlation Spectroscopy (DCS) can monitor CBF by tracking red blood cells. These measures can be combined to describe metabolism, a key indicator of tissue viability.

In this study we present the development and testing of a hybrid broadband NIRS/DCS neuromonitor. This system is novel in its ability to simultaneously acquire broadband NIRS and DCS signals, providing a truly real-time measure of metabolism. Narrow bandpass and notch filters have been incorporated to diminish light contamination between the two modalities, preferentially filtering out each source from the opposing detector, allowing for an accurate measure of ScO2, CBF, and metabolism. With a broadband NIRS/DCS system, a real-time measure of CBF and metabolism within the developing brain can aid clinicians in monitoring events that precede brain injury, ultimately leading to better clinical outcomes.

10054-29, Session 7

Lymphatic Imaging in unsedated infants and children

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Lymphatic malformation or trauma can cause serious complications. However, the role of the lymphatics in cardiovascular disease remains poorly understood in part to the limited ability to safely and routinely image the lymphatics, especially in the pediatric population where the risks of ionizing radiation and sedation are considered significant. We recently demonstrated the use of near-infrared fluorescence lymphatic imaging (NIRFLI) in the pediatric population in young subjects with primary lymphedema or postoperative chylothorax following surgery for congenital heart defects.

After informed consent of parents, subjects were enrolled into an IRB and FDA approved protocol. Following intradermal injection of indocyanine green (ICG) for uptake in the lymphatic plexus, the children were illuminated with diffuse light and ICG-laden lymph was imaged using a custom imaging system outfitted with night vision technology. Images were assessed for abnormal lymphatic anatomy, drainage patterns, and active contractile propulsion.

To date we have imaged 6 infants with postoperative chylothorax following open heart surgery. Chylothorax is manifested by the drainage of chyle in the pleural space and is thought to result from trauma to the thoracic duct. Three distinct drainage patterns were observed that provided evidence to guide surgical management. In the cause of a 21 month old male presenting with unilateral right arm and hand lymphedema at birth, NIRFLI visualized linear, well-defined lymphatics in the legs and arms with no abnormal anatomy as typically seen in our studies of lymphedematous adults. In

contrast, we visualized a lack for contractile activity was responsible for lymphedema.

10054-30, Session 7

Fibre-optic endoscope location through imaging of ballistic photons

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Optic fibre based endoscopes are increasingly used for imaging and sensing internally within the human body without navigational guidance of the miniaturised fibre probe. We demonstrate successful determination of endoscope location with centimetre accuracy in clinically relevant models, in a realistically lit environment, through the capture of ballistic photons with a time resolved single photon detector array.

In this prototype system, short (~100ps) laser pulses are transmitted from the tip of the endoscope at 785nm in the “optical window” where attenuation is less severe. Most of the photons that pass through tissue undergo much scattering from the disordered structures (flesh and bone) providing only low accuracy determination of the location of the light source. However ballistic photons are those that travel through a scattering medium in an almost straight line without scattering. Such ballistic photons exit the body sooner than the highly scattered light.

A camera based upon a 32 ? 32 array of Single Photon Avalanche Photodiode Detectors (SPADs) is used to image the small number of ballistic photons. The time resolution capabilities of such a single photon detector (50ps time bin resolution, 200ps jitter) allow observation of the photon arrival time, separating the ballistic photons from the highly scattered light, enabling accurate imaging of the endoscope location.

With appropriate filtering this compact packaged system is demonstrated in a normally lit room to determine endoscope location with centimetre accuracy in a whole ventilated ovine lung as well as tissue models including bone structure.

10054-31, Session 8

Ultrasound imaging using all-optical power and signal transfer in a catheter

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Smart medical catheters face a connectivity challenge. An example is found in ultrasound imaging where the supply of power at the distal end and the signal transmission requires many thin and fragile wires in order to keep the catheter thin and flexible and this leads to a relatively high cost of production. We have built a fully functional benchtop demonstrator that is immediately scalable to catheter dimensions, in which all electrical wires are replaced by just two optical fibers. We show signal transfer of synthetic aperture ultrasound images as well as photovoltaic conversion to supply all electronics. The absence of conductors provides excellent galvanic isolation as well as RF and MRI compatibility and the simple design utilizing off the shelf components holds a promise of cost effectiveness all of which may help translation of these advanced devices into the clinic.

We show photovoltaic conversion of 405 nm light to 45 V and 1.8 V by two blue LEDs as well as 200 MHz broad-band signal transfer using modulated 850 nm VCSEL light. Synthetic aperture ultrasound images are acquired at a frequency of 12 MHz with a collapse-mode capacitive-micromachined ultrasonic transducer. Bandwidth, noise level and dynamic range are nearly identical as shown in comparison of the images acquired with the optical link and its electrical equivalent. In conclusion, we have successfully demonstrated low-cost and scalable optical signal and power transmission for an ultrasound imaging system enjoying intrinsic RF / MRI compatibility and galvanic isolation.

10054-32, Session 8

Seeing laser scalpel: A novel monolithic high-power diode pumped Tm:YAG laser system at 2.02 μm with double-clad fiber combined OCT

Manuel Messner, Arne Heinrich, Clemens Hagen, Pantec Engineering AG (Liechtenstein); Karl Unterrainer, Technische Univ. Wien (Austria)

During the last two decades lasers have been well established in almost all fields of medicine. The strong water absorption around 3μm make Er:YAG lasers perfect candidates for all kinds of biological tissue interaction. However fibers, optics and coatings are not trivial and expensive, whereas alternatively lasers at around 2μm can be used. Although water absorption is weaker, standard fused silica fibers and optics are applicable, enabling a price competitive, biocompatible solution for minimally-invasive endoscopic surgery. Ideally the surgeon could see the underlying tissue prior being laser-ablated and OCT is the best detection method for this purpose. In order to combine the laser ablation with an OCT in a rugged fashion, e.g. with a double-clad fiber, where the laser is guided in the outer core of 200μm and the OCT is in the centre, a special laser is needed, which should be pulsed with high average power and pulse energy and fit into the 200μm fiber core. Existing Tm fiber lasers or flash-lamp pumped CTH lasers fail either due the pulse energy or the fiber diameter.

To fulfil these requirements, we report on a new monolithic high-power diode pumped Tm:YAG solid state laser at 2.02μm. The pulsed laser generates average output power and energy of up to 90W and 900mJ in 300μs pulses, respectively. The laser can be operated within a wide settings range, the compact and robust laser design in combination with commercial double-clad fiber couplers are likely making a “seeing scalpel” possible in the near future.

10054-33, Session 8

Compact system with handheld microfabricated optoelectronic probe for needle-based tissue sensing applications

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Tissue optical spectroscopy has been employed to detect human diseases, including cancers, by providing a quantitative assessment of tissue morphology and biochemical status in situ. To optically interrogate tissue sites within solid tissues, the distal end of a fiber-optic probe may be delivered to the site via a hollow needle. Though commonly employed in tissue spectroscopy, needle-based fiber-optic probes have several limitations, including size and spatial configurability. Further, fiber-optic probe based systems rely on bulky and expensive optical components, including optical sources and detectors, which could limit clinical utility.

Here, we present the design, development, and bench-top verification of an innovative compact clinical system including a miniaturized handheld optoelectronic sensor. The integrated sensor was microfabricated with die-level light-emitting diodes and photodiodes and fits into a 19G hollow needle (internal diameter: 0.75 mm), thereby replacing bulky optical components (e.g., fiber-optic probes, optical sources, and detectors) for optical sensing applications in solid tissues. Compact custom-designed

electronics coupled with system software control data acquisition. Bench-top studies on tissue-simulating phantoms have verified system performance relative to a fiber-optic based tissue spectroscopy system.

In addition to dramatically reducing system size and cost, the technology affords spatially configurable designs for optoelectronic light sources and detectors, thereby enabling customized sensing configurations that would be impossible to achieve with needle-based fiber-optic probes.

10054-34, Session 8

Comparison and use 3D scanners to improve the quantification of medical images (surface structures and volumes) during follow up of clinical (surgical) procedures

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It is difficult to obtain quantitative measurements as to surface areas and volumes from standard photos of the body of patients which is highly desirable for objective follow up of treatments in e.g. dermatology. plastic, esthetic and reconstructive surgery. Recently, 3-D scanner have become available to provide quantification.

Phantoms (3-D printed hand, nose and ear, colored bread sculpture) were developed to compared a range from low-cost (Kinect, Sense), medium (HP Sprout) to high end (Artec Spider, Vectra M3) scanners using different 3D imaging technologies, as to resolution, working range, surface color representation, user friendliness. The 3D scans files (STL, OBJ) were processed with Artec studio and GOM software as to deviation compared to the high resolution Artec spider scanner taken as 'golden' standard. The HP Spout, which uses a fringe projection, proved to be nearly as good as the Artec, however, needs to be converted for clinical use. Photogrammetry as used by the Vectra M3 scanner is limited to provide sufficient data points for accurate surface mapping however provides good color/structure representation. The low performance of the Sense is not recommended for clinical use.

The Artec scanner as successfully used to measure the structure/volume changes in the face after hormone treatment in transgender patients.

3D scanners can greatly improve quantitative measurements of surfaces and volumes as objective follow up in clinical studies performed by various clinical specialisms (dermatology esthetic and reconstructive surgery). New scanning technologies, like fringe projection, are promising for development of low-cost, high precision scanners.

10054-35, Session 8

Fibre Bragg grating manometry catheters for in-vivo monitoring of peristalsis

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The human gastrointestinal tract or 'gut' is one of the body's largest functional systems spanning up to 8 metres in length from beginning to end. It is formed of a series of physiologically different sections that perform the various functions required for the digestion of food, absorption of nutrients and water, and the removal of waste products. To enable the gut to perform correctly it must be able to transport digesta through each section at the appropriate rate, and any breakdown or malfunction of this transport mechanism can have severe consequences to on-going good health.

Monitoring motor function deep within the gut is challenging due to the need to monitor over extended lengths with high spatial resolution. Fiber Bragg grating (FBG) manometry catheters provide a ideal method of monitoring physiologically significant lengths of the gut in a minimally invasive fashion. Following the development by our group of the first viable FBG based manometry catheter we have undertaken a series of clinical investigations in the human esophagus, colon, stomach and small bowel. Each region presents its own technological challenge and has required a range of modifications to the basic catheter design. We present the design of these catheters and clinical results from over 100 in-vivo studies.

10054-40, Session 9

Volume measurement of the leg with the depth camera for quantitative evaluation of edema

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In a leg edema, it is necessary to care continuously. Therefore, volume measurement of the leg is important in the evaluation of edema. Recently, method for measurement by using a depth camera is proposed to obtain volume of the leg. However, it is difficult to use it as home care system because many depth cameras are expensive. In this paper, we propose a method of volume measurement leg using Microsoft Kinect, which is already spread as a game controller. Firstly, we scan the leg by Kinect with Kinect Fusion technique, and then we can obtain a point cloud of the leg in real time. Secondly, the point cloud is processed to remove unnecessary parts. To decide the range of measurement, we set the coordinate axis along the leg length. Finally, the volume value is obtained by voxelization. In the experiment, we measured the volume of the leg for three healthy students in the morning and evening during three days. In each measurement, the mean value and the coefficient of variation were obtained from five measured values. Consequently, the increase of volume was confirmed in all experiment from morning to evening. The coefficient of variation was within the range of 0.5% - 2.5%. It is known that the volume of leg is increased in the evening compared in the morning when the subjects are doing office work. Therefore we can conclude that our experimental results meet this expectation and the experimental system is practical to measure the leg edema.

10054-41, Session 9

Evaluation of endoscopic entire 3D image acquisition of the digestive tract using a stereo endoscope

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Because the view angle of the endoscope is narrow, it is difficult to get the whole image of the digestive tract at once. If there are more than two lesions in the digestive tract, it is hard to understand the 3D positional relationship among the lesions. Virtual endoscopy using CT is a present standard method to get the whole view of the digestive tract. Because the virtual endoscopy is designed to detect the irregularity of the surface, it cannot detect lesions that lack irregularity including early cancer. In this study, we propose a method of endoscopic entire 3D image acquisition of the digestive tract using a stereo endoscope. The method is as follows: 1) capture sequential images of the digestive tract by moving the endoscope, 2) reconstruct 3D surface pattern for each frame by stereo images, 3) estimate the position of the endoscope by image analysis, 4) reconstitute the entire image of the digestive tract by combining the 3D surface pattern. To confirm the validity of this method, we experimented with a straight

tube inside of which circles were allocated at equal distance of 20 mm. We captured sequential images and the reconstituted image of the tube revealed that the distance between each circle was 19.5 ± 1.0 mm (n=7). The results suggest that this method of endoscopic entire 3D image acquisition may help us understand 3D positional relationship among the lesions such as early esophageal cancer that cannot be detected by virtual endoscopy using CT.

10054-42, Session 9

Novel diffuse optics system for continuous tissue viability monitoring: extended recovery in vivo testing in a porcine flap model

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In reconstructive surgery, tissue perfusion is critical to the success of flaps created via free tissue transfer. In case of impending failure, implementing earlier detection of any vascular compromise would increase the chances of flap salvage. Current methods, which rely on infrequent and often subjective assessments such as flap color, temperature, and refill rate do not address the need for continuous objective monitoring and cannot assess instantaneous detection of failure.

A compact, low-cost, clinically-compatible monitoring system capable of automated, minimally-invasive, continuous, and quantitative assessment of tissue blood flow, hemoglobin concentration and tissue oxygenation is presented. The system employs diffuse correlation and reflectance spectroscopy for tissue sensing via fiber-optic patches for light delivery and collection, and software for continuous monitoring.

Here, we present results of continuous flap monitoring during an extended recovery (for 5 hours) using an in vivo porcine flap model. The latissimus dorsi muscle of a mature pig was isolated with only thoracodorsal vessels (artery and vein) remaining. Microsurgical anastomosis was subsequently performed. Fiber-optic patches were placed on the artery, and on the muscle and skin sides of the flap. In case flap failure was not observed within 5 hours, failure was simulated by occluding the vein. Artery occlusion followed after 30-minute monitoring of venous occlusion. Raw data was analyzed to quantitatively extract tissue parameters.

Initial results indicated that the system could assess flap viability in an objective and continuous manner. With proven performance, the compact form constructed with cost-effective components would make this system suitable for clinical translation.

10054-43, Session 9

Design and evaluation of a miniature laser speckle imaging device to assess gingival health

Caitlin Regan, Univ. of California, Irvine (United States) and Beckman Laser Institute (United States); Sean M. White, Bruce Y. Yang, Beckman Laser Institute (United States) and Univ. of California, Irvine (United States); Thair Takesh, Jessica Ho, Beckman Laser Institute and Medical Clinic (United States); Cherie Wink, Concorde Career College (United States); Petra Wilder-Smith, Bernard Choi, Univ. of California, Irvine (United States) and Beckman Laser Institute and Medical Clinic (United States)

Current methods used to assess gingivitis are qualitative and subjective. We hypothesized that gingival perfusion measurements could provide a quantitative metric of disease severity. We constructed a compact laser speckle imaging (LSI) system that could be mounted in custom-made oral molds. Rigid fixation of the LSI system in the oral cavity enabled measurement of blood flow in the gingiva. In vitro validation performed in controlled flow phantoms demonstrated that the compact LSI system had comparable accuracy and linearity compared to a conventional bench-top LSI setup. In vivo validation demonstrated that the compact LSI system was capable of measuring expected blood flow dynamics during a standard post-occlusive reactive hyperemia and that the compact LSI system could be used to measure gingival blood flow repeatedly without significant variation in measured blood flow values (CV <10%). Finally, compact LSI system measurements were collected from the interdental papilla of n=9 subjects and compared to a clinical assessment of gingival bleeding on probing. A statistically significant correlation (? = -0.53; p < 0.005) was found between these variables, indicating that quantitative gingival perfusion measurements performed using our system may aid in the diagnosis and prognosis of periodontal disease.

10054-44, Session 9

Intraoperative detection of parathyroid gland perfusion during endocrine surgeries

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As many as 80,000 patients a year in the US undergo thyroidectomies or parathyroidectomies for diseased glands. About 21% of these surgeries result in disruption of blood supply to health parathyroid glands, which, if unaddressed, may result in long-term hypocalcemia. Surgeons need to know as soon as possible whether or not the blood supply to a parathyroid gland has been disrupted, as this informs their decision on whether or not to excise and reimplant the gland. There is a non-trivial failure rate involved in this transplantation process, and in the absence of an objective gold-standard surgeons often rely on subjective visual inspection in making this decision. Here we present Laser Speckle Imaging as a real-time objective method to assess parathyroid viability. Our device consists of a 785 nm laser source and a near-infrared camera with a zoom lens, positioned above the surgical field with an articulated arm. With the laser diffusing light onto the tissue, the camera acquires images which are processed in real-time and displayed on a monitor. These speckle contrast images are then averaged and the relative difference in speckle contrast between the parathyroid gland and surrounding thyroid tissue is calculated and correlated with the surgeon's assessment of viability. Preliminary findings from in vivo measurement of 9 diseased glands show 100% agreement with the surgeon when taking a greater than 5% relative difference to indicate devascularization. This device has the potential to be used as an intraoperative tool for assessing parathyroid viability.

10054-36, Session 10

3D endoscopic imaging using structured illumination technique for surgical guidance and assessment

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(United States); Jin U. Kang, Johns Hopkins Univ. (United States)

Surgeons have been increasingly relying on minimally invasive surgical guidance techniques not only to reduce surgical trauma but also to achieve accurate and objective surgical risk evaluations. A typical minimally invasive surgical guidance system provides visual assistance in two-dimensional anatomy and pathology of internal organ within a limited field of view. In this work, we propose and implement a structure illumination endoscope to provide a simple, inexpensive 3D endoscopic imaging to conduct high resolution 3D imagery for use in surgical guidance system. The system is calibrated and validated for quantitative depth measurement in both calibrated target and human subject. The system exhibits a depth of field of 20 mm, depth resolution of 0.2mm and a relative accuracy of 0.1%. The demonstrated setup affirms the feasibility of using the structured illumination endoscope for depth quantization and assisting medical diagnostic assessments

10054-37, Session 10

Structured illumination microscopy as a diagnostic tool for nephrotic disease

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Nephrotic disease is a group of debilitating and sometimes lethal diseases affecting kidney function, specifically the loss of ability to retain vital proteins in the blood while smaller molecules are removed through filtration into the urine. Treatment routes are often dictated by microscopic analysis of kidney biopsies. Podocytes within the glomeruli of the kidney have many interdigitating projections (foot processes), which form the main filtration system. Nephrotic disease is characterised by the loss of this tightly interdigitating substructure and its observation by electron microscopy (EM) is necessitated as these structures are typically 250-500nm wide, with 40nm spacing. Diagnosis by EM is both expensive and time consuming; it can take up to one week to complete the preparation, imaging, and analysis of a single sample.

We propose structured illumination microscopy (SIM) as an alternative, optical diagnostic tool. Our results show that SIM can resolve the structure of fluorescent probes tagged to podocin, a protein localised to the periphery of the podocyte foot processes. Three-dimensional podocin maps were acquired in healthy tissue and tissue from patients diagnosed with two different nephrotic disease states; minimal change disease and membranous nephropathy. These structures correlated well with EM images of the same structure. Preparation, imaging, and analysis could be achieved in several hours. Additionally, the volumetric information of the SIM images revealed morphological changes in disease states not observed by EM.

This evidence supports the use of SIM as a diagnostic tool for nephrotic disease and can potentially reduce the time and cost per diagnosis.

10054-38, Session 10

An optoacoustic guide with augmented reality for precision breast-conserving surgery

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Breast-conserving surgery is a well-accepted breast cancer treatment. However, it is still challenging for the surgeon to accurately localize the tumor during the surgery. Also, the guidance provided by current methods is 1 dimensional distance information, which is indirect and not intuitive. Therefore, it creates problems on a large re-excision rate, and a prolonged surgical time. To solve these problems, we have developed a fiber-delivered optoacoustic guide (OG), which mimics the traditional localization guide wire and is preoperatively placed into tumor mass, and an augmented reality (AR) system to provide real-time visualization on the location of the tumor with sub-millimeter variance. By a nano-composite light diffusion sphere and light absorbing layer formed on the tip of an optical fiber, the OG creates an omnidirectional acoustic source inside tumor mass under pulsed laser excitation. The optoacoustic signal generated has a high dynamic range (~ 58dB) and spreads in a large apex angle of 320 degrees. Then, an acoustic radar with three ultrasound transducers is attached to the breast skin, and triangulates the location of the OG tip. With an AR system to sense the location of the acoustic radar, the relative position of the OG tip inside the tumor to the AR display is calculated and rendered. This provides direct visual feedback of the tumor location to surgeons, which will greatly ease the surgical planning during the operation and save surgical time. A proof-of-concept experiment using a tablet and a stereo-vision camera is demonstrated and 0.25 mm tracking variance is achieved.

10054-39, Session 10

Terahertz spectroscopy of the proteinuria in the patients with nephropathy

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Spectroscopic analysis in terahertz-band has been utilized by the biochemistry community and it yields some structural information. Analyses of structures of the peptides or relatively complex proteins provide functional information. Vibrational modes of proteins lay in the terahertz frequency range. Protein spectra are dominated by ensembles of hydrogen bonds and collective modes. So terahertz spectroscopy is sensitive to the protein-water biochemical environment. Nephropathy is one of the common metabolic diseases which severely affect many organs in the human-body. It involves perturbation of carbohydrate, fat and protein metabolisms. The proteinuria is the complex solution mixed with the different protein molecules. The terahertz response of these proteins in the proteinuria depends on the norm modes of the protein molecules. In this research, we focused on the patients with nephropathy who had suffered from proteinuria. The urines with different concentrations of proteins were simultaneously measured by terahertz time-domain spectroscopy and 24-hour urinary protein measurements. The terahertz frequency absorption and refraction index were obtained. The terahertz dielectric responses based on different protein concentrations were analyzed. The comparison between the dielectric spectrum and the medical results of proteinuria were made in order to establish their correlations. Although other materials such as mineral salts are also exits in the urines, but their influence on the terahertz responses is less than that by the protein molecules. Overall, this research of the proteinuria by terahertz spectrum can contribute to better understand urine-protein's complex interaction with water and the diagnostics in nephropathy.

Conference 10055:
Optics and Biophotonics in Low-Resource Settings III

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10055-1, Session 1

Inkjet-printed paper surface enhanced Raman spectroscopy (SERS) sensors: portable, low cost diagnostics for microRNA biomarkers

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Diagnostic devices for rural or developing regions without access to advanced laboratory equipment require improvements in the time, cost, and technical expertise required for sensitive, lab based assays. Current designs for portable diagnostic devices primarily utilize lateral flow immunoassays that have limited sensitivity and multiplexibility. To improve upon current lateral flow diagnostics, we have designed a sensor capable of providing rapid and portable identification of multiple genomic targets, specifically microRNAs. MicroRNA (miRNA) provide a promising set of biomarkers to replace proteins and antibodies for a range of disease conditions from cancer to HIV and dengue fever. Our group pioneered production of inkjet printable SERS sensors and developed a single step microfluidic assay for nucleic acids. This work illustrates development of an integrated and fully inkjet-printed paper device for miRNA using a combination of SERS and a parallelizable displacement assay. Results show successful application of the displacement assay on printable nanoparticle substrates that will allow for rapid, simple, customizable fabrication of sensors for multiple miRNA sequences. Further, we show the potential for simultaneous detection of the multiplexed output from the displacement assay using inkjet-printed paper SERS devices.

10055-2, Session 1

Quartz-enhanced photo-acoustic spectroscopy for breath analyses

Jan Conrad Petersen, Mikael O. Lassen, Danish Fundamental Metrology Institut (Denmark)

The use of spectroscopy to analyze molecules in exhaled breath is a promising tool for medical diagnostics. Various spectroscopic techniques for this purpose have been reported in the literature. The techniques must be able to measure amount of substances (biomarkers) at the ppm and ppb levels as well as minor relative changes in these levels. In addition, the simultaneous measurement of a number of biomarkers is highly desirable since only one is rarely sufficient to decisively diagnose a disease. An innovative photoacoustic (PA) sensing technique is proposed and shown to be a very promising technology. Ideally sensors are expected to be cheap, portable, miniaturized, automated stand-alone devices, sensitive and species selective. In order to fulfil these requirements we present a sensor based on quartz-enhanced photoacoustic spectroscopy (QEPAS). The sensor consists of two acoustic coupled micro-resonators (mRs) with an off-axis 20 kHz quartz tuning fork (QTF). The acoustically coupled mR system is optimized based on finite element simulations and experimentally verified. The QEPAS sensor is pumped resonantly by either a tuneable cw diode laser or a nanosecond pulsed single-mode mid-infrared optical parametric oscillator (MIR OPO), tuneable from 3.1 μm to 3.5 μm with a resolution band-width of 1 cm-1. The suitability of the sensor is demonstrated by recording spectra of both individual molecules (CH4, (CH3)2CO, CH3OH, SO2,....) as well as complex mixtures of various molecules. The individual molecules as well as the mixtures are interesting for health diagnostics.

10055-3, Session 1

Smartphone-assisted multiplex nutrition deficiency diagnostics: multicolored quantification of inflammation marker, iron and vitamin A status

Zhengda Lu, David Erickson, Cornell Univ. (United States)

Vitamin A and iron deficiency are common malnutrition affecting billions of people worldwide. However, in infrastructure limited settings, access to blood vitamin A and iron status test is limited because of the complexity and cost of traditional diagnostic methods. Direct measurements of vitamin A and iron level is not easy to perform, and it is necessary to measure approximate marker for obtaining vitamin A and iron deficiency status. Measurement of inflammatory marker is also necessary because the vitamin A and iron level are altered by inflammation status. Here we introduced a multiplex rapid point-of-care (POC) diagnostic devices that simultaneously characterize three markers relevant to vitamin A, iron and inflammation status: retinol binding protein 4, ferritin and C-reactive protein with lateral flow immunoassay test strips. Level of retinol binding protein 4, ferritin and C-reactive protein are indicated by excitation intensity of fluorescence tags with three different colors. The test can be done within 15 minutes and a complete sample-answer quantitative results of vitamin A, iron and inflammation status level can be obtained with assists of a smartphone and an external device. We also demonstrated the device is able to perform colorimetric analysis on single test area. which gives the device potential to perform more tests simultaneously at the same time.

10055-4, Session 1

Cloud-based processing of multi-spectral imaging data

Amir Bernat, Frank J. Bolton, Reuven Weiser, David Levitz, MobileODT Ltd. (Israel)

Multi-spectral imaging is an imaging modality in which images are captured at multiple wavelengths, followed by computationally intensive data analysis. As a result, multi-spectral data analysis is done locally, requiring among others, reliable power supply and skilled users, which limits deployment and hindes algorithmic refinements scalability. Recently, a low-cost, portable multi-spectral imaging system built around a smartphone was developed for use in low-resource settings. In such systems, efficient data analysis and communication with the user is challenging. To address this challenge, a novel cloud based backend system is used for data analysis that communicates with MobileODT's production image portal as well as the phone running Android application. An Amazon Web Service EC2 server running Python and OpenCV packages is set up to perform multi-spectral data analysis in near real time. Captured multi-spectral data is uploaded to the image portal, which in turn is passed to the EC2 server for automatic analysis, results are then pushed back to the portal and to the user in the field within a short time. Typical output include augmented images, image analytics and clinical insights.

This novel backend architecture allowed deploying multi-spectral devices in several places around the globe for use by practitioners as part of medical tests. Data analysis turnaround time is under 10 minutes. Moreover, this architecture provides a rapid, scalable deployment solution with automated centralized updates for data analysis algorithms.

10055-5, Session 1

Demosaiced pixel super-resolution in digital holography for multiplexed computational color imaging on a chip

Yichen Wu, Yibo Zhang, Wei Luo, Aydogan Ozcan, Univ. of California, Los Angeles (United States)

Digital holographic on-chip microscopy achieves large space-bandwidth-products (e.g., >1 billion) by making use of pixel super-resolution techniques. To synthesize a digital holographic color image, one can take three sets of holograms representing the red (R), green (G) and blue (B) parts of the spectrum and digitally combine them to synthesize a color image. The data acquisition efficiency of this sequential illumination process can be improved by 3-fold using wavelength-multiplexed R, G and B illumination that simultaneously illuminates the sample, and using a Bayer color image sensor with known or calibrated transmission spectra to digitally demultiplex these three wavelength channels. This demultiplexing step is conventionally used with interpolation-based Bayer demosaicing methods. However, because the pixels of different color channels on a Bayer image sensor chip are not at the same physical location, conventional interpolation-based demosaicing process generates strong color artifacts, especially at rapidly oscillating hologram fringes, which become even more pronounced through digital wave propagation and phase retrieval processes. Here, we demonstrate that by merging the pixel super-resolution framework into the demultiplexing process, such color artifacts can be greatly suppressed. This novel technique, termed demosaiced pixel super-resolution (D-PSR) for digital holographic imaging, achieves very similar color imaging performance compared to conventional sequential R,G,B illumination, with 3-fold improvement in image acquisition time and data-efficiency. We successfully demonstrated the color imaging performance of this approach by imaging stained Pap smears. The D-PSR technique is broadly applicable to high-throughput, high-resolution digital holographic color microscopy techniques that can be used in resource-limited-settings and point-of-care offices.

10055-6, Session 1

Applications of optical spectroscopy in low-resource settings *(Invited Paper)*

Matthew D. Keller, Sebastian Wachsmann-Hogiu, Intellectual Ventures Lab. (United States)

Optical spectroscopy techniques possess many characteristics that have made them attractive as potential alternatives to traditional analyses in several fields of health and agriculture. These advantages include the ability to perform in vivo or in situ non-destructive measurements, which can produce objective results in near-real time. These qualities could be particularly advantageous in low-resource settings that lack the infrastructure and skilled labor to carry out many traditional lab-based analyses. To date, a major limiting factor in their implementation has been the cost of such devices; however, recent advancements have brought the cost of several modalities, such as fluorescence, near infrared reflectance, and Raman spectroscopy down to levels that are approachable by potential end-users in low-resource settings, including health clinics and government extension programs. At Intellectual Ventures Laboratory, we have investigated several promising applications. These include examining soil nutrients for providing guidance to smallholder farmers, examining the quality and makeup of fertilizers that are often adulterated or mislabeled, and examining matrices like grains and milk for both nutritional information and the presence of toxins. We will present our findings to support these applications, as well as address the major challenges in maintaining sufficient performance of the technology with low cost, ruggedized versions of the relevant hardware.

10055-7, Session 2

Yeast viability and concentration measurements using lens-free computational microscopy and machine learning

Alborz Feizi, Yibo Zhang, Alon Greenbaum, Alexander Guziak, Michelle C. Luong, Raymond Yan Lok Chan, Univ. of California, Los Angeles (United States); Brandon R. Berg, Univ. of Michigan (United States); Wei Luo, Michael Wu, Yichen Wu, Aydogan Ozcan, Univ. of California, Los Angeles (United States)

Research laboratories and the industry rely on yeast viability and concentration measurements to adjust fermentation parameters such as pH, temperature, and pressure. Beer-brewing processes as well as biofuel production can especially utilize a cost-effective and portable way of obtaining data on cell viability and concentration. However, current methods of analysis are relatively costly and tedious. Here, we demonstrate a rapid, portable, and cost-effective platform for imaging and measuring viability and concentration of yeast cells. Our platform features a lens-free microscope that weighs 70 g and has dimensions of 12 x 4 x 4 cm. A partially-coherent illumination source (a light-emitting-diode), a band-pass optical filter, and a multimode optical fiber are used to illuminate the sample. The yeast sample is directly placed on a complementary metal-oxide semiconductor (CMOS) image sensor chip, which captures an in-line hologram of the sample over a large field-of-view of >30 mm^2. The hologram is transferred to a touch-screen interface, where a trained support vector machine (SVM) model classifies yeast cells stained with methylene blue as live or dead and measures cell viability as well as concentration. We tested the accuracy of our platform against manual counting of live and dead cells using fluorescent exclusion staining and a bench-top fluorescence microscope. Our regression analysis showed no significant difference between the two methods (P > 0.05) within a concentration range of 3.0x10^5 to 3.0x10^6 cells/mL. This compact and cost-effective yeast analysis platform will enable automatic quantification of yeast viability and concentration in field settings and resource-limited environments.

10055-8, Session 2

Cancer recognition using a smartphone and deep learning

Theodore V. Hromadka III, Integrity Applications, Inc. (United States); Niels H. Olson, U. S. Navy (United States)

All radical prostatectomy specimens with Gleason 3+4 carcinoma were identified in the NMCSD pathology archive and 55 were randomly selected, scanned with an Aperio CS-1 whole slide imaging system (e-Slide manager version 11.2) at 20x magnification, and annotated by a team of pathologists who identified regions of carcinoma (by Gleason grade), perineural invasion, extraprostatic extension, positive margins, and peri-tumoral inflammation. These images were chipped into 256x256 pixel images, and classified as either cancer or not cancer, according to the pathologist annotations. Neural networks of various topologies based on AlexNet and GoogLeNet were trained with this supervised image data and evaluated using the Caffe deep learning framework and cuDNN machine learning library. Variations in image magnification levels, chip size, color scale, and network parameters were explored. The maximum specificity achieved with this approach was 91%; an expert human is expected to be 95% accurate. This network and the caffe framework were ported to a smartphone running iOS as a proof of concept, with software modifications to accommodate the smartphone's limited resources. Following this experiment, the image recognition network was re-trained with expanded datasets such as CAMELYON16 to reach cases beyond prostate histology. The latest results will be discussed.

10055-9, Session 2

A survey of supervised machine learning models for mobile-phone based pathogen identification and classification

Hatice Ceylan Koydemir, Steve W. Feng, Kyle Liang, Rohan Nadkarni, Derek Tseng, Parul Benien, Aydogan Ozcan, Univ. of California, Los Angeles (United States)

Giardia lamblia is a waterborne parasite affecting millions of people each year worldwide, even in developed countries, and causes a disease known as giardiasis, which results in diarrhea, abdominal cramps, and bloating. Although conventional pathogen detection methods used in water analysis laboratories offer high sensitivity and specificity, they are time consuming, and need experts to operate bulky equipment and analyze the samples. Here we present a field-portable and cost-effective smartphone-based waterborne pathogen detection platform that can automatically classify Giardia cysts using machine learning. Our platform has a technology-readiness-level of 5+ and enables detection and quantification of Giardia cysts in one hour, including sample collection, labeling, filtration, and automated counting steps. We evaluated the performance of three prototypes using Giardia-spiked water samples from different sources (e.g. reagent-grade, tap, non-potable, and pond water samples). We populated a training database with statistical features of >30,000 cysts and estimated our detection sensitivity and specificity using 20 different classifier models, including decision trees, nearest neighbor classifiers, support vector machines (SVMs), and ensemble classifiers, with respect to their speed of training and classification, and predicted accuracies. Among them, quadratic SVM, fine Gaussian SVM, and bagged-trees were the most promising classifier types with accuracies of ~ 87.9%, 89%, and 89.8%, respectively; we selected the latter as our preferred classifier for the detection and enumeration of Giardia cysts that are imaged using our mobile-phone fluorescence microscope. Without the need for any experts, this field-portable pathogen detection platform can be a useful tool for water quality monitoring in resource-limited-settings.

10055-10, Session 2

A cost-effective smartphone-based antimicrobial susceptibility test reader for drug resistance testing

Steve W. Feng, Derek Tseng, Dino Di Carlo, Omai B. Garner, Aydogan Ozcan, Univ. of California, Los Angeles (United States)

Antimicrobial susceptibility testing (AST) is commonly used for determining microbial drug resistance, but routine testing, which can significantly reduce the spread of multi-drug resistant organisms, is not regularly performed in resource-limited and field-settings due to technological challenges and lack of trained diagnosticians. We developed a portable cost-effective smartphone-based colorimetric 96-well microtiter plate (MTP) reader capable of automated AST without the need for a trained diagnostician. This system is composed of a smartphone used in conjunction with a 3D-printed opto-mechanical attachment, which holds a set of inexpensive light-emitting-diodes and fiber-optic cables coupled to the 96-well MTP for enabling the capture of the transmitted light through each well by the smartphone camera. Images of the MTP plate are captured at multiple exposures and uploaded to a local or remote server (e.g., a laptop) for automated processing/analysis of the results using a custom-designed smartphone application. Each set of images are combined to generate a high dynamic-range image and analyzed for well turbidity (indicative of bacterial growth), followed by interpretative analysis per plate to determine minimum inhibitory concentration (MIC) and drug susceptibility for the specific bacterium. Results are returned to the originating device within ~1 minute and shown to the user in tabular form. We demonstrated the capability of this platform using MTPs prepared with 17 antibiotic drugs targeting Gram-negative bacteria and tested 82 patient isolate MTPs of Klebsiella pneumoniae, achieving well turbidity accuracy of 98.19%, MIC accuracy of 95.15%, and drug susceptibility interpretation accuracy of 99.06%, meeting the FDA defined criteria for AST.

10055-11, Session 3

Innovative healthcare is in the palm of your hand *(Keynote Presentation)*

Luke Lee, Univ. of California, Berkeley (United States)

In this talk, I will present how to see the world's healthcare crisis and the fundamental problems of current medicine in a grain of iSAND (interactive e-Science, Arts, Nanomedicine, and Digital healthcare), and find solutions in nature for preventive medicine and healthy environment. Since the future of healthcare is in the palm of our hands, a few examples of creative healthcare innovations will be discussed along with the vision of smart digital healthcare in both developing and developed countries: smart mobile integrated molecular diagnostic systems (iMDx) for personalized precision medicine and integrated microphysiological analytics platforms (iMAPs) for toxicology, drug discovery, and regenerative medicine. The rapid and accurate smart mobile iMDx comprises three key elements of precision medicine on chip: ultrafast photonic PCR for the early detection of DNA and RNA biomarkers in blood, signal amplifications of protein markers, and a self-contained sample preparation from whole blood on chip, which allows a sample-to-answer readout platform with smart analytics. The progress on patient-specific iPSCs-based iMAPs, pancreatic islets and mini-brains in silicone for molecular pathogenesis will be discussed along with the vision of BIGHEART: preventive medicine via precision engineering medicine.

10055-12, Session 3

Measurement and evaluation of digital cervicography programs in two cervical cancer screening camps in Kenya

Curtis Peterson, Jonah W. Mink, David Levitz, MobileODT Ltd. (Israel)

Cervical cancer disproportionately affects women living in low- and middle-income countries. To address this global crisis, many governments and NGOs have implemented community-based screening and treatment programs at outreach camps. Here, high volumes of patients are able to access care: screening and diagnosis followed by immediate treatment of precancerous lesions onsite. However, monitoring and evaluation (M&E) of these efforts presents challenges, since each event typically relies on a different health workforce, and refers patients to different facilities for follow up and advanced care. To address these challenges, a digital imaging intervention was deployed at two large scale screening camps in Kenya. Trained nurses screened women using a connected low-cost mobile colposcope built around a smartphone. A decision support job aid was integrated into the app controlling the device, guiding nurses and recording their diagnosis and treatment decisions. Aggregating the data from the job aid allowed M&E of the screening camp in real-time. In this paper, the M&E data from 2 different screening camps in Kenya (Nairobi and Nyeri County) are compared. Differences in the patient screening times, treatment rates, and individual nurse statistics were all documented through the job aid allowing for much improved epidemiological information following outreach events thus enabling targeted program improvements and provider training. Reporting data from screening camps were also shared online via public web pages, facilitating broader dissemination of health needs in specific Kenyan communities, and sparking conversations with regional stakeholders about local disease burden.

10055-13, Session 3

Cost-effective and label-free holographic biosensor for detection of Herpes Simplex Virus

Aniruddha Ray, Ha Ho, Mustafa Daloglu, Avee Torres, Univ. of California, Los Angeles (United States); Euan McLeod, The Univ. of Arizona (United States); Aydogan Ozcan, Univ. of California, Los Angeles (United States)

Herpes is one of the most widespread sexually transmitted viral diseases. Timely detection of Herpes Simplex Virus (HSV) can help prevent the rampant spreading of the virus. Current detection techniques such as viral culture, immuno-assays or Polymerase-Chain-Reaction, are time extensive and require expert handling. Here we present a field-portable, easy-to-use, and cost-effective biosensor for the detection of HSV based on holographic imaging. The virus is first captured from a target solution onto specifically developed substrates, prepared by coating glass coverslips with HSV-specific antibodies, and imaged using a lensfree holographic microscope. Several light-emitting-diodes (LEDs), coupled to multi-mode optical-fibers, are used to illuminate the sample containing the viruses. A micro-controller is used to activate the LEDs one at a time and in-line holograms are recorded using a CMOS imager placed immediately above the substrate. These sub-pixel shifted holograms are used to generate a super-resolved hologram, which is reconstructed to obtain the phase and amplitude images of the viruses. The signal of the viruses is enhanced using self-assembled PEG-based nanolenses, formed around the viral particles. Based on the phase information of the reconstructed images we can estimate the size of the viral particles, with an accuracy of ± 11 nm, as well as quantify the viral load. The limit-of-detection of this system is estimated to be <500 viral copies per 100 μL sample volume that is imaged over 30 mm^2 field-of-view. This holographic microscopy based biosensor is label-free, cost-effective and field-portable, providing results in 2 hours, including sample preparation and imaging time.

10055-14, Session 3

Evaluation of anemia diagnosis based on elastic light scattering

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Currently, one-third of humanity is still suffering from anemia. In China the most common forms of anemia are iron deficiency and Thalassemia minor. Differentiating these two is the key to effective treatment. Iron deficiency is caused by malnutrition and can be cured by iron supplementation. Thalassemia is a hereditary disease in which the hemoglobin ? chain is lowered or absent. Iron therapy is not effective, and there is evidence that iron therapy may be harmful to patients with Thalassemia. Both anemias can be diagnosed using red blood cell morphology: Iron deficiency presents a smaller mean cell volume compared to normal cells, but with a wide distribution; Thalassemia, meanwhile, presents a very small cell size and tight particle size distribution. Several researchers have proposed diagnostic indices based on red cell morphology to differentiate these two diseases. However, these indices lack sensitivity and specificity and are constructed without statistical rigor. Using multivariate methods we demonstrate a new classification method based on red cell morphology that diagnoses anemia in a Chinese population with enough accuracy for its use as a screening method. We further demonstrate a low cost instrument that precisely measures red cell morphology using elastic light scattering. This instrument is combined with an automated analysis program that processes scattering data to report red cell morphology without the need for user intervention. Despite using consumer-grade components, when comparing our experimental results with gold-standard measurements, the device can still achieve the high precision required for sensing clinically significant changes in red cell morphology.

10055-15, Session 4

Towards practical implementation of biophotonics-based solutions for cost-effective monitoring of food quality control *(Invited Paper)*

Igor Meglinski, Alexey Popov, Alexander Bykov, Univ. of Oulu (Finland)

Biophotonics-based diagnostic and imaging modalities have been widely used in various applications associated with the non-invasive imaging of the internal structure of a range biological media from a range of cells cultures to biological tissues. With the fast growing interest in food securities there remains strong demand to apply reliable and cost effective biophotonics-based technologies for rapid screening of freshness, internal defects and quality of major agricultural products. In current presentation the results of application of optical coherence tomography (OCT) and encapsulated optical bio-sensors for quantitative assessment of freshness of agricultural products, such as meat and sea foods, are presented, and their further perspectives are discussed.

10055-17, Session 4

Doppler MR-OCT angle optimization and depth characterization for flow detection and velocity measurement

Sean O'Gorman, National Univ. of Ireland, Galway (Ireland)

Multiple reference optical coherence tomography (MR-OCT) is a new compact optical imaging device based on a recirculating reference arm scanning optical delay. This technology promises to be a robust, cost-effective semi-solid state design capable of integrating with next generation mobile devices. The re-circulating optical delay utilizes a voice coil actuator and a partial mirror to build up a multiple order interference pattern, which can be simultaneously detected with each sweep of the actuator. Our group has recently demonstrated the capability of this method to detect and measure flow using Doppler signal analysis. The MR-OCT system operates at 1310nm with an axial spatial resolution of ~26 μm and an axial scan rate of 600 Hz. Initial studies have shown a displacement-sensitivity of ~20 nm to ~120 nm for the first 1 to 9 orders of reflections respectively, using a mirror as an ideal reflector. The corresponding minimum resolvable velocity for each of these orders is ~2.3 μm/s and ~15 μm/s respectively. In this work, the angle-dependence of the Doppler signal and flow measurements in agar flow phantoms are investigated to examine the depth dependence of Doppler measurements in scattering media. The feasibility of in-vivo Doppler MR-OCT for providing liveness detection in spoof and biometric applications is also investigated.

10055-18, Session 4

A fully automated colorimetric sensing device using smartphone for biomolecular quantification

Sibasish Dutta, Tezpur Univ. (India)

In the present work, the use of smartphone for colorimetric quantification of biomolecules has been demonstrated. As a proof-of-concept, BSA protein and carbohydrate have been used as biomolecular sample. BSA protein and carbohydrate at different concentrations have been treated with Lowry's

reagent and Anthrone's reagent respectively . The change in color of the reagent-treated samples at different concentrations have been recorded with the camera of a smartphone in combination with a custom designed optomechanical hardware attachment. This change in color of the reagent-treated samples has been correlated with color channels of two different color models namely RGB (Red Green Blue) and HSV (Hue Saturation and Value) model. In addition to that, the change in color intensity has also been correlated with the grayscale value for each of the imaged sample. A custom designed android app has been developed to quantify the bimolecular concentration and display the result in the phone itself. The obtained results have been compared with that of standard spectrophotometer usually considered for the purpose and highly reliable data have been obtained with the designed sensor. The device is robust, portable and low cost as compared to its commercially available counterparts. The data obtained from the sensor can be transmitted to anywhere in the world through the existing cellular network. It is envisioned that the designed sensing device would find wide range of applications in the field of analytical and bio-analytical sensing research.

10055-19, Session 4

Imaging cytometry in a plastic ultra-mobile system

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Flow cytometers are fundamental instruments in biomedical sciences as they are used in routine analysis, like blood cell count, or in more complex assays in cell biology, immunology, oncology, etc. Current commercial flow cytometers have reached table-top size with very high sensitivity and throughput and are capable of performing very complex analysis, however they have a high cost and a very limited portability. Translating this technology to a cost-effective and portable hardware could open new perspectives in biomedicine enabling sophisticated analysis also in remote or resource-limited environments.

In this work we propose a cost-effective and highly-portable plastic prototype that can be interfaced with a cell phone to implement an optofluidic imaging cytometry platform.

It is based on a PMMA microfluidic chip that is fabricated by microinjection moulding (exploiting reconfigurable inserts) and is afterwards bonded by femtosecond laser welding.

The chip fits inside an opto-mechanical case, built through a 3D printer, which suits onto the mobile phone. The 3D case is designed to hold all the optical components necessary to perform the fluorescence imaging of the samples flowing inside the plastic microfluidic chip.

In our cytometry platform we exploit both the LED and the CMOS camera from the cell phone, the first as the excitation light and the camera to capture fluorescence microscopic images of flowing micro-beads solutions. During measurements, these images are quickly processed on the cell phone using a custom developed application that provide a value of micro-beads concentration

10055-20, Session 5

Towards practical cost-effective lens-free imaging

Abdulkadir Yurt, Richard Stahl, Geert Vanmeerbeeck, Ziduo Lin, Murali Jayapala, Andy Lambrechts, IMEC (Belgium)

Lens-free in-line Holographic Microscopy (LHM) is a promising imaging technique for many life science and industrial applications. However the system miniaturization and cost reduction without compromising imaging performance remains a challenge for most field applications in low-resource settings.

Iterative phase-retrieval (IPR) methods have been developed to overcome the deficiencies of the in-line holography, namely its twin-image problem. We have previously demonstrated that a multi-wavelength illumination, based on a single-mode fiber optics, complemented by an IPR algorithm can considerably improve image quality and resolution. Despite the versatility of fiber optics, providing means to combine and spatially filter multiple coherent sources, their cost and bulkiness hinder their practical use in field applications.

Herein, we demonstrate a cost-effective LHM system based on spatially distributed multi-wavelength illumination. Our method eliminates the need for precision optical-mechanical parts (such as pinholes, beam-splitters, or kinematic mounts) and relies solely on robust optical hardware and reconstruction software co-design to overcome the challenges associated with IPR using spatially distributed discrete sources. Prior to IPR, an in-house developed hologram flat-fielding and registration are applied to the raw images eliminating fixed pattern noise from the distinct sources. Furthermore the reconstruction algorithm is modified to account for the unique reference waves associated with each source. The resulting hardware simplicity enables low-cost and scalable LHM implementation without sacrificing image quality and system performance. The system reaches sub-micron resolution across the full field-of-view of 16mm2. We have evaluated the LHM system in a number of experiments, ranging from industrial to bio-medical applications.

10055-21, Session 5

Development of add-on kit for scanning confocal microscopy

Kaikai Guo, Guoan Zheng, Univ. of Connecticut (United States)

Scanning confocal microscopy is a standard choice for many fluorescence imaging applications in basic biomedical research. It is able to produce optically sectioned images and provide acquisition versatility to address many samples and application demands. However, scanning a focused point across the specimen limits the speed of image acquisition. As a result, scanning confocal microscope only works well with stationary samples. Researchers have performed parallel confocal scanning using digital-micromirror-device (DMD), which was used to project a scanning multi-point pattern across the sample. The DMD based parallel confocal systems increase the imaging speed while maintaining the optical sectioning ability. In this paper, we report the development of an add-on kit for high-speed and low-cost confocal microscopy. By adapting this add-on kit to an existing regular microscope, one can convert it into a confocal microscope without significant hardware modifications. Compared with current DMD-based implementations, the reported approach is able to recover multiple layers along the z axis simultaneously. It may find applications in wafer inspection and 3D metrology of semiconductor circuit. The dissemination of the proposed add-on kit under $1000 budget could also lead to new types of experimental designs for biological research labs, e.g., cytology analysis in cell culture experiments, genetic studies on multicellular organisms, pharmaceutical drug profiling, RNA interference studies, investigation of microbial communities in environmental systems, and etc.

10055-22, Session 5

High resolution computational on-chip imaging of biological samples using sparsity constraint

Yair Rivenson, Univ. California, Los Angeles (United States); Chris Wu, Hongda Wang, Yibo Zhang, Aydogan Ozcan, Univ. of California, Los Angeles (United States)

Microscopic imaging of biological samples such as pathology slides is one of the standard diagnostic methods for screening various diseases, including cancer. These biological samples are usually imaged using traditional optical microscopy tools; however, the high cost, bulkiness and limited imaging throughput of traditional microscopes partially restrict their deployment in resource-limited settings. In order to mitigate this, we previously demonstrated a cost-effective and compact lens-less on-chip microscopy platform with a wide field-of-view of >20-30 mm^2. The lens-less microscopy platform has shown its effectiveness for imaging of highly connected biological samples, such as pathology slides of various tissue samples and smears, among others. This computational holographic microscope requires a set of super-resolved holograms acquired at multiple sample-to-sensor distances, which are used as input to an iterative phase recovery algorithm and holographic reconstruction process, yielding high-resolution images of the samples in phase and amplitude channels. Here we demonstrate that in order to reconstruct clinically relevant images with high resolution and image contrast, we require less than 50% of the previously reported nominal number of holograms acquired at different sample-to-sensor distances. This is achieved by incorporating a loose sparsity constraint as part of the iterative holographic object reconstruction. We demonstrate the success of this sparsity-based computational lens-less microscopy platform by imaging pathology slides of breast cancer tissue and Papanicolaou (Pap) smears.

10055-23, Session 5

High resolution flat lensless phase imager

Manon Rostykus, Christophe Moser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Compact lensless optical imaging systems are mainly applied in microscopy for biological samples. Indeed, they are well suited as imagers inside cell incubators because of their compact size (high throughput with many imagers per incubator) and sub-micron resolution over cm2. Several presented systems uses digital holography to recover phase and amplitude of a sample. To avoid speckle, most of the systems utilize incoherent light sources positioned many centimeters away from the sample and camera chip to record interferograms. Moreover, in order to increase the resolution of the result, the sources are shifted mechanically by a small amount to obtain subpixel shifted interferograms on the camera to retrieve a higher resolved interferograms.

Here, we demonstrate a compact illumination system for a high resolution lensless phase imager. The device is composed of a side illumination system that uses a prism onto which a photopolymer film is laminated on one side. An array of light sources is set along one side of the prism and several analog hologram gratings are recorded in the photopolymer film to redirect the light out of the prism with different specific beam angles. The sample can thus be illuminated with different directions and a camera placed behind the sample records the inline holograms. For each illumination direction, the wavelength of the sources is changed to obtain slightly different illumination direction of the sample and obtain several subpixel shifted digital inline holograms which are then processed in a pixel super resolution algorithm to retrieve a highly resolved hologram. The stack of highly resolved holograms is then processed in a reconstruction using Gerchberg-Saxton and tomographic type algorithm. The quantitative phase and amplitude of the samples are reconstructed with high resolution.

The side illumination system effectively folds one axis (z axis) which creates a flat lensless imaging system.

10055-24, Session 5

Super-resolution through out-of-focus imaging in lens-based microscopy

Hongda Wang, Wei Luo, Zoltan Gorocs, Laurent A. Bentolila, Aydogan Ozcan, Univ. of California, Los Angeles (United States)

The limited space-bandwidth-product of microscopy systems in general forces users to sacrifice either resolution or field-of-view (FOV). Here we introduce a wide-field and high-resolution imaging method that uses a stack of out-of-focus images of the specimen to increase the space-bandwidth-product of lens-based microscopes. Although modern microscope objective-lenses are designed for high-resolution imaging and can achieve a relatively large FOV, often the active area of the imager chip sets a limitation. To best utilize the full field-of-view of an objective-lens in our microscope, we first added a demagnification camera adaptor (e.g., 0.35?) to match the CCD sensor chip active area to the FOV of a 10X objective-lens (~5 mm2) that has an NA of 0.3. This demagnification, while increasing the FOV, downgrades the image resolution and results in pixelation. We illustrate that this spatial undersampling can be overcome through an iterative pixel super-resolution algorithm that uses a stack of out-of-focus images of the sample to restore a high-resolution image across a large FOV. We demonstrated the success of this approach using a resolution test-target and showed that our technique reduces the number of measurements required to achieve the same effective space-bandwidth-product using e.g., lateral scanning and digital stitching of different FOVs. Phase retrieval capability of this approach is also demonstrated by reconstructing unstained Papanicolaou (Pap) smear samples without the need for phase-contrast objective-lenses. This technique might be useful to maximize the throughput of lens-based optical imaging systems and inspire new microscopy designs that utilize auto-focusing steps to increase resolution.

10055-25, Session 5

Disposable cartridge biosensor platform for portable diagnostics *(Invited Paper)*

Yusuf Yaras, Koç Univ. (Turkey); Onur Cakmak, Columbia Univ. (United States); Ali Gunduz, Gokhan Saglam, Selim Olcer, Ibrahim Baris, Koç Univ. (Turkey); Fehmi Çivitci, Istanbul Technical Univ. (Turkey); Goksen Yaralioglu, Ozyegin Univ. (Turkey); Hakan Urey, Koç Univ. (Turkey)

We developed a biosensor platform suitable for low-cost portable diagnostics. We performed direct mechanical coagulation time measurement using plasma and whole blood in a disposable cartridge containing microfluidic channels. Two types of sensors have been developed, one sensor is based on MEMS cantilever chip mounted in the fluidic channel and the other sensor is based on a pair of optical fibers (one is movable) crossing the microfluidic channel. For both types of sensors, actuation of the cantilever or the moving fiber is achieved using an electromagnet and the readout is optical. Since both the actuation and sensing are remote, there are not electrical connections and the cartridge is easily made disposable. The compact reader unit contains light sources, photodetectors, electromagnet, heater, electronics, and microprocessor. MEMS based platform has better sensitivity but optomechanical alignment is a challenge and measurements with whole blood were not possible due to scattering of light by the red blood cells. Fiber based platform ease the optomechanical tolerances and easy to manufacture and allows measurement in whole blood. The proposed sensor platform is tested on control plasma samples and human whole blood samples for activated-Partial-Thromboplastin-Time (aPTT) measurements. Control plasma test results matched with the manufacturer's datasheet. aPTT tests were successfully carried out with human whole blood samples and provided repeatable test results.

10055-26, Session 6

Multi-scale silica structured substrates for improved point of care detection *(Invited Paper)*

Michelle Khine, Univ. of California, Irvine (United States)

Leveraging self-assembled silica (SiO2) micro- and nanostructures via pre-stressed thermoplastic (shrink-wrap) film relaxation, we demonstrate uniform and robust fluorescence signal enhancements on these substrates. We demonstrate that the SiO2 structures can be used to increase the fluorescence signal by over 100x and importantly, significantly improve the signal to noise ratio. We demonstrate improved detection of fluorescently labeled proteins, DNA, and even improved limits of detection for the human immunodeficiency virus type 1 (HIV-1) p24 antigen. We determined the mechanism responsible for the dramatic increases in signal intensity and finally leverage this for bright field assays as well.

10055-27, Session 6

Fusion of lens-free microscopy and mobile-phone microscopy images for high-color-accuracy and high-resolution pathology imaging

Yibo Zhang, Chris Wu, Yun Zhang, Aydogan Ozcan, Univ. of California, Los Angeles (United States)

Digital pathology and telepathology require imaging tools with high-throughput, high-resolution and accurate color reproduction. Lens-free on-chip microscopy based on digital in-line holography is a promising technique towards these needs, as it offers a wide FOV (>20 mm2) and high resolution with a compact, low-cost and portable setup. Color imaging has been previously demonstrated by combining reconstructed images at three discrete wavelengths in the red, green and blue parts of the visible spectrum i.e., RGB combination method. However, this RGB combination method is subject to color distortions.

To improve the color performance of lens-free microscopy for pathology imaging, we here present a wavelet-based color fusion imaging framework, termed “digital color fusion microscopy” (DCFM), which digitally fuses together a grayscale lens-free microscope image taken at a single wavelength and a low-resolution and low-magnification color-calibrated image taken by a lens-based microscope, which can simply be mobile phone based cost-effective microscope. We show that the imaging results of an H&E stained breast cancer tissue slide with the DCFM technique come very close to a color-calibrated microscope using a 40x objective lens with 0.75 NA. Quantitative comparison showed a significant (~ 2x) reduction in the mean color distance using the DCFM method compared to the RGB combination method, while also preserving the high resolution features of the lens-free microscope. Due to the cost-effective and field-portable nature of both lens-free and mobile-phone microscopy techniques, their combination through the DCFM framework could be useful for digital pathology and telepathology applications, in low-resource and point-of-care settings.

10055-28, Session 6

Near-infrared fluorescence imaging with a mobile phone

Pejhman Ghassemi, U.S. Food and Drug Administration (United States); Bohan Wang, Univ. of Maryland, College Park (United States); Jianting Wang, Quanzeng Wang, U.S. Food and Drug Administration (United States); Yu Chen, Univ. of Maryland, College Park (United States); T. Joshua Pfefer, U.S. Food and Drug Administration (United States)

Mobile phone cameras employ sensors with near-infrared (NIR) sensitivity, yet this capability has not been exploited for biomedical purposes. Removing the IR-blocking filter from a phone-based camera opens the door to a wide range of techniques and applications for inexpensive, point-of-care biophotonic imaging and sensing. This study provides proof of principle for one of these modalities – phone-based NIR fluorescence imaging. An imaging system was assembled using a 780 nm light source along with excitation and emission filters with 800 nm and 825 nm cut-off wavelengths, respectively. Indocyanine green (ICG) was used as an NIR fluorescence contrast agent in an ex vivo rodent model, a resolution test target and a 3D-printed, tissue-simulating vascular phantom. Raw and processed images for red, green and blue pixel channels were analyzed for quantitative evaluation of fundamental performance characteristics including spectral sensitivity, detection linearity and spatial resolution. Mobile phone results were compared with a scientific CCD. The spatial resolution of CCD system was consistently superior to the phone, and green phone camera pixels showed better resolution than blue or green channels. The CCD exhibited similar sensitivity as processed red and blue pixels channels, yet a greater degree of detection linearity. Raw phone pixel data showed lower sensitivity but greater linearity than processed data. Overall, both qualitative and quantitative results provided strong evidence of the potential of phone-based NIR imaging, which may lead to a wide range of applications from cancer detection to glucose sensing.

10055-29, Session 6

Development and testing of a homogenous multi-wavelength LED light source

Frank J. Bolton, Amir Bernat, MobileODT Ltd. (Israel); Steven L. Jacques, Oregon Health & Science Univ. (United States); David Levitz, MobileODT Ltd. (Israel)

Multispectral imaging of human tissue is a powerful method that allows for the quantification of scattering and absorption parameters of the tissue and for the differentiation of tissue types or the identification of pathology. This method requires imaging at multiple wavelengths and then fitting the measured data to a model based on light transport theory. Earlier, a mobile phone based multi-spectral imaging system was developed to image the uterine cervix from the colposcopy geometry, outside the patient’s body at a distance of 200-300 mm. Such imaging of a distance object has inherent challenges, as bright and homogenous illumination are required. Several solutions addressing this problem were developed, with varied degrees of success. In this paper, several multi-spectral illumination setups were developed and tested for brightness and uniformity. All setups were specifically designed with low cost in mind, utilizing a printed circuit board with surface-mounted LEDs. The three setups include: LEDs illuminating the target directly, LEDs illuminating focussed by a 3d printed miniature lens array, and LEDs coupled to a mixing lens and focusing optical system. In order to compare the illumination uniformity and intensity performance of the different configurations, a custom low cost illumination quality test tool was developed using 3d printed parts, low cost electronics, and open source software tools. Test results are presented, and various tradeoffs between the three system configurations are discussed.

10055-30, Session 6

Development of mobile phone based transcutaneous billirubinometry

Alexander Dumont, Brandon Harrison, Zachary T. McCormick, Temple Univ. (United States); Nishant Ganesh Kumar, Vanderbilt Univ. (United States); Chetan A. Patil, Temple Univ. (United States)

Extreme or prolonged neonatal jaundice (hyperbilirubinemia) can result

in permanent neurological impairment and even death. In the developing world, risk factors that increase the risk of neurodevelopmental impairment, such as sepsis, malnutrition, and certain genetic conditions are common. Treatment can be simple but identification of at-risk infants through visual screening is unreliable. Infants in the US are routinely screened prior to hospital discharge using transcutaneous bilirubinometry (TcB), a non-invasive technique based on diffuse reflectance. In low-resource settings such as rural sub-Saharan Africa, TcB devices are not available to traditional birth attendants and doctors; however, it is increasingly common for these personnel to carry mobile phones equipped with a camera and flash. We have previously demonstrated the feasibility of TcB utilizing the built-in camera and flash of the mobile phone. Here, we report the results of a Monte Carlo model of diffuse reflectance in neonatal skin with a mobile phone, along with a redesign of the snap-on optical assembly to accommodate an altered optical path. We will discuss a revised set of methods for image analysis, along with updated results from clinical studies in healthy newborns which correlate mobile-phone based measurements of TcB with corresponding serum bilirubin levels. These results will lead to a discussion of feasibility and limitations for mobile-phone based TcB.

10055-31, Session PSun

Design of mobile phone based total internal reflection fluorescence microscope for real-time fluorescence detection

Taerim Yoon, Dong-Myeong Shin, Kyujung Kim, Pusan National Univ. (Korea, Republic of)

The purpose of the cell culture is to look for a response to a variety of drugs and analysis biological phenomena via the studies of the cell. A cell incubator provides a suitable environment for the growth of biological samples through the control of the temperature and CO2 concentration. In order to maintain this environment, an incubator is closed for biological samples are not affected by external environment. Therefore, A closed cell incubator is limited to real-time observation. In order to solve this problem, we designed the mobile phone based total internal reflection fluorescence microscope(TIRF-MoPM). The TIRF-MoPM is used for the purpose of real time obtaining high resolution cell fluorescence image in a cell incubator. The microscope uses the prims in order to generate the total internal reflection phenomenon, and we designed the optimal arrangement of other optics through the 3D printer. Therefore, the TIRF-MoPM is installed at a miniature cell incubator, and it makes long-term measurements without inserting samples into or removing samples from a cell incubator, and transmits data in real-time to an external device over a wireless network. Using the MoPM, we were able to successfully capture the various reaction of the cell in accordance with the drug injection in real time. We are convinced that the TIRF MoPM is a useful device for capturing the high resolution image of biological samples in real-time and long-term, and can make it possible to carry out live observations via wireless communications regardless of location.

10055-32, Session PSun

Design of ultra-compact optical system for disposable epidural spinal endoscope

Hyeon Jin Bang, Byung Jun Park, Byung Yeon Kim, Young Jae Won, Seung Rag Lee, Osong Medical Innovation Foundation (Korea, Republic of)

We have presented the plastic based ultra-compact aspheric lens for disposable epidural spinal endoscope. We have also showed the analysis of the stray light distribution on the image plane using optical illumination system design software. The optical system consists of the aspheric lens with TTL of 1.4mm. The effective length and field of view (FOV) is 0.66mm and 90 degrees. The distortion of the optical system is below 25%. The curves of modulation transfer function (MTF) are higher than 0.3 at 80 line pairs/mm (lps/mm) in image space. For the analysis of stray light, we assumed that the 98 percent of incident light is absorbed inside lens barrel and the rest is scattered on the inner surfaces of the lens barrel. The average value of stray light is 0.16% in the image intensity. The maximum stray light and minimum stray light of the proposed optical system is 0.57% and 0.0005% in the image intensity, respectively. The effective transmission rate of the proposed optical system is 89.6%.

10055-33, Session PSun

Computational laser intensity stabilisation for organic molecule concentration estimation in low-resource settings

Shahid A. Haider, Farnoud Kazemzadeh, Alexander Wong, Univ. of Waterloo (Canada)

An ideal laser is a useful tool for the analysis of biological systems. In particular, the polarisation property of lasers can allow for the concentration of important organic molecules in the human body, such as proteins, amino acids, lipids, and carbohydrates, to be estimated.

However, lasers do not always work as intended and there can be effects such as mode hopping and thermal drift that can cause time-varying intensity fluctuations. The causes of these effects can be from the surrounding environment, where either an unstable current source is used or the temperature of the surrounding environment is not temporally stable. This intensity fluctuation can cause bias and error in typical organic molecule concentration estimation techniques. In a low-resource setting where cost must be limited and where environmental factors, like unregulated power supplies and temperature, cannot be controlled, the hardware required to correct for these intensity fluctuations can be prohibitive.

We propose a method for computational laser intensity stabilisation that uses Bayesian state estimation to correct for the time-varying intensity fluctuations from electrical and thermal instabilities without the use of additional hardware. This method will allow for consistent intensities across all polarization measurements for accurate estimates of organic molecule concentrations. Validation will be done by measuring the concentration of sucrose dissolved in distilled water using an unstabilised HeNe laser and comparing those measurements to estimates using the computational stabilisation method.

10055-34, Session PSun

Comparing an FFT filter for multiple reference optical coherence tomography (MR-OCT) with an Chebychev and an elliptic filter

Kai Neuhaus, Sergey A. Alexandrov, National Univ. of Ireland, Galway (Ireland); Roshan I. Dsouza, Compact Imaging, Inc. (United States); Sean O'Gorman, National Univ. of Ireland, Galway (Ireland); Paul M. McNamara, Josh Hogan, Carol J. Wilson, Compact Imaging, Inc. (United States); Martin J. Leahy, National Univ. of Ireland, Galway (Ireland); Hrebesh Molly Subhash, National Univ. of Ireland Galway (Ireland)

Portable and low-cost optical coherence tomography (OCT) is increasingly used to improve the accuracy of point-of-care applications.

The increasing research efforts to use photonics integrated circuits (PIC) is without doubts the future for highest packaging densities in miniature optical systems.

MR-OCT is another technology that is using the advantages of well known

CD/DVD-ROM technology to build miniaturized and low-cost OCT systems and may be more readily available before PICs reach their full potential.

For MR-OCT it is essential to separate the the multiple signals originating from the multiple reflections of the partial mirror in the reference arm of Michelson interferometer.

An image analysis on MR-OCT scans is performed for filter types such as a Chebychev2 and an elliptic filter, but also an FFT filter with Gaussian window is discussed.

The SNR is compared on a variety of test signals generated with a mirror in the sample arm at different attenuation levels and the CNR is used to compare the image quality.

10055-35, Session PSun

The reliability and accuracy of estimating heart-rates from RGB video recorded on a consumer grade camera

Adam T. Eaton, Vinoin D. Vincely, Paige Lloyd, Kurt Hugenberg, Karthik Vishwanath, Miami Univ. (United States)

Video Photoplethysmography (VPPG) is a numerical technique to process standard RGB video recording of exposed human skin in order to estimate the subjects' heart rate (HR). Given that behavioral research routinely involves video recording participants, VPPG algorithms may prove beneficial for use in these studies as it naturally offers the ability to extract physiological endpoints such as HR, respiratory rate and/or heart rate variability in subjects. Although several earlier reports have shown good correlations between HR values obtained using VPPG algorithms to those obtained using the gold-standard electrocardiograph (ECG), others have reported that degree of these correlations were dependent on other variables including subject motion and ambient lighting. Here, we study the impact of natural and unavoidable subject motion during video recordings obtained during routine psychological experiments on the extracted HR using VPPG algorithms. Two previously published VPPG algorithms were employed to process data recorded using a standard consumer-grade video camera from four human subjects. Subjects also underwent simultaneous ECG measurements during the video recording. Video recordings were obtained for two conditions for each subject – at baseline and then after 5 minutes of deep-breathing, or 5 minutes of stair climbing. All studies were conducted with appropriate IRB approvals. Recorded videos were processed to extract the HR from VPPG with and without facial feature tracking software (Computer Vision MATLAB® toolbox). Results from these experiments and the impact of controlling for small movement artifacts to ensure spatial uniformity in processing the video timeframe will be presented and discussed.

10055-36, Session PSun

Portable, low-resource smartphone based frequency doubling perimetry using virtual reality

Karam A. Alawa, Bascom Palmer Eye Institute (United States)

No abstract available.

10055-37, Session PSun

Design and test of a smartphone-based intraoral imaging system for oral cancer detection

Ross Uthoff, Hsiang Nan Cheng, Chih-Yu Huang, College of Optical Sciences, The Univ. of Arizona (United States); Petra Wilder-Smith D.D.S., Beckman Laser Institute and Medical Clinic (United States); Praveen Birur, KLE Society's Institute of Dental Sciences (India); Moni A. Kuriakose, Mazumdar Shaw Cancer Center (India); Mary Platek, Roswell Park Cancer Institute (United States); Rongguang Liang, College of Optical Sciences, The Univ. of Arizona (United States)

Oral cancer is a health crisis in many low-resource communities. The proposed device is a novel implementation of autofluorescence imaging on a smartphone platform providing inexpensive early detection of cancerous conditions in the oral cavity. The geometry of this device allows imaging of the base of the tongue and cheek pockets, which are areas of increased risk. The system implements custom optics to decrease the smartphone camera's field of view and external LEDs to excite the tissue fluorescence response. Future work will focus on implementation and testing of the device in clinical and low-resource settings.

10056-39, Session 1

Label-free hyperspectral dark-field microscopy for quantitative scattering imaging of tissue-mimicking phantoms

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The diffuse reflectance spectroscopy (DRS) based on point sources and detectors combined with a physics based model has enabled quantitative tissue optical measurements to correlate the measurement results with standard pathological and clinical interpretations. However, this point probe technique has a limitation in achieving real-time spatial information at a larger region of interest towards effective diagnosis and surgical treatments of diseased tissues where the spatial information of the tissue scattering properties is critical. We have developed a quantitative dark-field scatter imaging technique to measure the local scattering coefficient of biomedical scattering phantoms and human breast tissues. Performance of the microscope was validated by the wavelength-dependent back scattering signals from well-defined scatter phantoms along with the results from the Monte Carlo photon transport model. Our ongoing effort involves quantitative mapping of local scattering coefficients of breast tissue lumpectomies to enable label-free quantitative optical biopsy for the on-site detection of tumor margins.

10056-40, Session 1

Polydimethyl siloxane tissue-mimicking phantoms for quantitative optical medical imaging standards

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We report techniques to build and characterize solid tissue-mimicking phantoms with polydimethyl siloxane (PDMS) polymers capable of tuning physical and optical characteristics including scattering, absorption, and photoacoustic response properties. Controlled inclusion, in a uniform or a layered pattern, of scattering nanoparticles and microbeads incorporated with light-absorbing molecules enables phantoms for their applications in multiple imaging platforms. This technique allows for the validation of optical properties towards SI-traceable, cross-validated standards in multiple measurement platforms. Backscatter confocal microscopy and high resolution optical coherence tomography characterize the shape, distribution, density, and refractive index of individual particles and layered structures. This information allows for building physics-based analytical or photon transport simulation models to quantify and validate the results from macroscopic and mesoscopic measurement modalities including double integrating sphere system, time-resolved diffuse transmittance and reflectance measurements, sub-diffuse spatial frequency domain imager, and hyperspectral dark-field microscopy.

10056-41, Session 1

Broadband spectral measurements of diffuse optical properties by a double integrating sphere instrument at the National Institute of Standards and Technology

Paul Lemaillet, Jeeseong Hwang, David W. Allen, National Institute of Standards and Technology (United States)

Biological phantoms, made from scattering and absorbing materials in a liquid or in solid matrix, are used to mimic the optical properties of tissues in the development, characterization and maintenance of optical imaging instruments. Scales for reflectance and transmittance are maintained by the National Institute of Standards and Technology (NIST). Such a scale is needed for volume scattering of diffuse materials and this is currently being addressed by the development of a Double integrating sphere Optical Scattering Instrument (DOSI) reference instrument at NIST. The basic system and methods have been described in previous papers. In this presentation we will report measurements of the optical properties of liquid and solid phantoms samples under visible to near infrared broadband illumination provided by NIST's DOSI. The measured of diffuse hemispherical reflectance and transmittance are analyzed using a custom inversion algorithm of the adding-doubling routine providing a detailed uncertainty budget.

10056-1, Session 2

Additive 3D bioprinting and mesoscopic molecular imaging *(Invited Paper)*

Xavier Intes, Guohao Dai, David T. Corr, Rensselaer Polytechnic Institute (United States)

No abstract available.

10056-2, Session 2

3D printing of microtube network in solid phantom to simulate tissue oxygenation and perfusion

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Tissue-simulating phantoms with interior vascular network may facilitate traceable calibration and quantitative validation of many medical optical devices. However, a solid phantom that reliably simulates tissue oxygenation and blood perfusion is still not available. This paper presents a new method to fabricate hollow microtubes for blood vessel simulation in solid phantoms. The fabrication process combines ultraviolet (UV) rapid prototyping technique with fluid mechanics of a coaxial jet flow. Polydimethylsiloxane (PDMS) and a UV-curable polymer are mixed at the designated ratio and extruded through a coaxial needle device to produce a coaxial jet flow. The extruded jet flow is quickly photo-polymerized by

ultraviolet (UV) light to form vessel-simulating solid structures at different sizes ranging from 700 ?m to 1000 ?m. Microtube structures with adequate mechanical properties can be fabricated by adjusting material compositions and illumination intensity. Curved, straight and stretched microtubes can be formed by adjusting the extrusion speed of the materials and the speed of the 3D printing platform. To simulate vascular structures in biologic tissue, we embed vessel-simulating microtubes in a gel wax phantom of 10 cm x10 cm x 5 cm at the depth from 1 to 2 mm. Bloods at different oxygenation and hemoglobin concentration levels are circulated through the microtubes at different flow rates in order to simulate different oxygenation and perfusion conditions. The simulated physiologic parameters are detected by a tissue oximeter and a laser speckle blood flow meter respectively and compared with the actual values. Our experiments demonstrate that the proposed 3D printing process is able to produce solid phantoms with simulated vascular networks for potential applications in medical device calibration and drug delivery studies.

10056-3, Session 2

3D printed optical phantoms and deep tissue imaging for in vivo applicationsincluding oral surgery

Brian Z. Bentz, Purdue Univ. (United States); Vaibhav Gaind, KLA-Tencor (United States); Kevin J. Webb, Purdue Univ. (United States)

Recent 3D printing results for optical imaging applications are presented. Biomedical applications require customizable and often complex objects for testing, evaluation, and calibration. There is therefore high demand for what have become known as tissue-simulating “phantoms”. We describe versatile and inexpensive fabrication methods for optical phantoms, including fused deposition modeling and stereolithography. 3D printing allows improved design of the phantom geometry, compared to previous methods which relied on molds, and in addition allows exact placement of complex inhomogeneities. We demonstrate design of the phantom and tuning of the optical parameters using Mie theory. We show mouse phantoms with deeply imbedded and anatomically realistic inhomogeneities, and we image these phantoms using optical diffusion tomography (ODT) and fluorescence optical diffusion tomography (FODT). ODT is a deep tissue optical imaging modality that allows extraction of absorption and scattering parameters as a function of 3D space. FODT involves the additional extraction of the fluorescence yield and lifetime images, providing in vivo information useful for studying molecular processes through targeted fluorescence agents. Finally, we describe a new method for optically imaging arteries in the roof of the mouth to assist with surgery, and demonstrate the method with a 3D printed phantom of the human mouth.

10056-4, Session 2

NIRS-based hematoma detector performance testing with molded and 3D-printed phantoms

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Near-infrared spectroscopy (NIRS) has emerged as a low-cost, portable approach for rapid, point-of-care detection of hematomas caused by traumatic brain injury. As a new technology, there is a need to develop standardized test methods for objective, quantitative performance evaluation of these devices. Towards this goal, we have developed and studied two types of phantom-based testing approaches. The first involves 3D-printed phantoms incorporating hemoglobin-filled inclusions. Phantom layers representing specific cerebral tissues were printed using photopolymers doped with varying levels of titanium oxide and black resin. The accuracy, precision and spectral dependence of printed phantom optical properties were validated using spectrophotometry. The phantom also includes a hematoma inclusion insert which was filled with a hemoglobin solution. Oxygen saturation levels were modified by adding sodium dithionite at calibrated concentrations. The second phantom approach involves molded silicone layers with a superficial region – simulating the scalp and skull – comprised of removable layers to vary hematoma size and depth, and a bottom layer representing brain matter. These phantoms were tested with both a commercial hematoma detector and a custom NIRS system to optimize their designs and validate their utility in performing inter-device comparisons. The effects of hematoma depth, diameter, and height, as well as tissue optical properties and biological variables including hemoglobin saturation level and scalp/skull thickness were studied. Results demonstrate the ability to quantitatively compare NIRS device performance and indicate the promise of using 3D printing to achieve phantoms with realistic variations in tissue optical properties for evaluating biophotonic device performance.

10056-5, Session 2

3D-printed biomimetic cerebrovascular phantoms for biophotonic imaging and spectroscopy

Pejhman Ghassemi, U.S. Food and Drug Administration (United States); Andrew Depkon, Marquette Univ. (United States) and U.S. Food and Drug Administration (United States); Jianting Wang, U.S. Food and Drug Administration (United States); Yu Chen, Univ. of Maryland, College Park (United States); T. Joshua Pfefer, U.S. Food and Drug Administration (United States)

Near-infrared (NIR) imaging and spectroscopy approaches represent emerging clinical tools for cerebral applications such as oximetry monitoring, perfusion assessment and cancer localization. Current methods for performance testing of these biophotonic technologies typically involve the use of tissue-simulating phantoms comprised of simple geometries such as planar layers and cylindrical or spherical inclusions. In order to enable benchtop assessment of new cerebral diagnostic systems in a manner that is more indicative of clinical performance, we have investigated the ability of three-dimensional (3D) printing to fabricate cerebral phantoms with biomimetic geometries that include vascular networks. The initial phases of this work involved converting an existing segmented cerebral CT image volume into a printable form and evaluating approaches for generating 3D-printed phantoms with biologically realistic optical properties. Techniques for cleaning residual uncured photopolymer from tortuous channels were assessed. Validation of printed phantom geometry based on micro-CT imaging indicated that printed channel diameters were consistently smaller than nominal values. Preliminary testing of the cerebral vascular phantoms was achieved by: (a) injecting hemoglobin solutions and imaging with a custom hyperspectral oximetry system and (b) injecting a mixture of hemoglobin and indocyanine green dye and performing measurements with a custom NIR fluorescence imaging system. Results demonstrated the utility of 3D-printed biomimetic phantoms and indicate their promise for improving performance assessment of optical systems for medical applications.

10056-6, Session 3

Solid tissue phantoms for NIR water fraction studies

Gordon T. Kennedy, Griffin R. Lentsch, Rolf B. Saager, Anthony J. Durkin, Beckman Laser Institute and Medical Clinic (United States)

Changes in local tissue water content can arise in the form of edema resulting from infection or trauma. Such shifts may have important diagnostic or prognostic implications. To this end, various groups have developed optical instrumentation to quantify tissue water fraction. However, investigations that quantitatively characterize the sensitivity of these technologies have lagged due to a dearth of controllable model systems that enable methodical variation in water fraction. We have developed solid tissue mimicking phantoms that enable us to simulate the changes in tissue water that might typify burn associated edema. We describe phantoms that incorporate a near infrared dye to produce a controllable absorption peak in the second O-H overtone of water at 970nm. Phantoms were prepared in a clear polydimethylsiloxane (PDMS) base using titanium oxide scattering particles. Absorption was provided by adding an infrared dye dissolved in acetone, which has a broad absorption feature with peak absorption at 970nm.

Measurements of the absorption and reduced scattering coefficients were performed on phantoms having a range of dye concentrations corresponding to physiologically relevant water fractions using the techniques of spatial frequency domain imaging, frequency domain photon migration and inverse adding doubling for wavelengths spanning 450nm – 1000nm. The stability of the phantom properties was characterized over a period of 6 months. These robust phantoms having separately tunable scattering and absorption are useful for the calibration and characterization of diffuse optical tissue imaging systems in the near infrared.

10056-7, Session 3

Visibility of solid and liquid fiducial markers used for image-guided radiation therapy on optical coherence tomography: an esophageal phantom study

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Radiation therapy (RT) is used in operable and inoperable esophageal cancer patients. Endoscopic ultrasound-guided fiducial marker placement allows improved translation of the disease extent on endoscopy to computed tomography (CT) images used for RT planning and enables image-guided RT. However, microscopic tumor extent at the time of RT planning is unknown. Endoscopic optical coherence tomography (OCT) is a high-resolution (10-30μm) imaging modality with the potential for accurately determining the longitudinal disease extent. Visibility of fiducial markers on OCT is crucial for integrating OCT findings with the RT planning CT.

We investigated the visibility on OCT (NinePoint Medical, Inc.) of 13 commercially available solid (Visicoil, Gold Anchor, Flexicoil, Polymark, and QLRAD) and liquid (BioXmark, Lipiodol, and Hydrogel) fiducial markers of different diameter. We designed and manufactured a set of dedicated Silicone-based esophageal phantoms to perform imaging in a controlled environment. The esophageal phantoms consist of several layers with different TiO2 concentrations to simulate the scattering properties of a typical healthy human esophagus. Markers were placed at various depths (0.5, 1.1, 2.0, and 3.0mm).

OCT imaging allowed detection of all fiducial markers and phantom layers. The signal to background ratio was 6-fold higher for the solid fiducial markers than the liquid fiducial markers, yet OCT was capable of visualizing all 13 fiducial markers at all investigated depths. We conclude that RT fiducial markers can be visualized with OCT. This allows integration of OCT findings with CT for image-guided RT.

10056-8, Session 3

Development and validation of a biologically realistic tissue-mimicking material for photoacoustics and other bimodal optical-acoustic modalities

William C. Vogt, Congxian Jia, Keith A. Wear, Brian S. Garra, T. Joshua Pfefer, U.S. Food and Drug Administration (United States)

Recent years have seen rapid development of hybrid optical-acoustic imaging modalities with broad applications in research and clinical imaging, including photoacoustic tomography (PAT), photoacoustic microscopy, and ultrasound-modulated optical tomography. Tissue-mimicking phantoms are an important tool for objectively and quantitatively simulating in vivo imaging system performance. However, no standard tissue phantoms exist for such systems. One major challenge is the development of tissue-mimicking materials (TMMs) that are both highly stable and possess biologically realistic properties. To address this need, we have explored the use of various formulations of PVC plastisol (PVCP) based on varying mixtures of several liquid plasticizers. We developed a custom PVCP formulation with optical absorption and scattering coefficients, speed of sound, and acoustic attenuation that are tunable and tissue-relevant. This TMM can simulate different tissue compositions and offers greater mechanical strength than hydrogels. Optical properties of PVCP samples with varying composition were characterized using integrating sphere spectrophotometry and the inverse adding-doubling method. Acoustic properties were determined using a broadband pulse-transmission technique. To demonstrate the utility of this bimodal TMM, we present PVCP phantoms designed to assess PAT image quality, which were imaged using a custom combined PAT-ultrasound imaging system. Phantoms included simple, idealized image quality phantoms and heterogeneous breast-mimicking phantoms with layered fat and parenchyma morphology. Results illustrate the use of this TMM for developing phantoms suitable for image quality testing and investigation of complex tissue effects. In the future, this TMM may be broadly utilized for performance evaluation of optical, acoustic, and hybrid optical-acoustic imaging systems.

10056-9, Session 3

Comparison of the temperature accuracy between smart phone based and high-end thermal cameras using a temperature gradient phantom

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Recently, low cost smart phone based thermal cameras are being considered to be used in a clinical setting for monitoring physiological temperature responses such as: body temperature change, local inflammations, perfusion changes or (burn) wound healing. These thermal cameras contain uncooled micro-bolometers with an internal calibration check and have a temperature resolution of 0.1 degree. For clinical applications a fast quality measurement before use is required (absolute temperature check) and quality control (stability, repeatability, absolute temperature) should be performed regularly. Therefore, a calibrated temperature phantom has been developed based on thermistor heating on both ends of a black coated metal strip to create a controllable temperature gradient from room temperature (~20 C) up to 100 C. The absolute temperatures on the strip are determined with software controlled 3 PT-100 sensors using lookup tables. In this study 3 FLIR-ONE cameras and one high end camera were checked with this temperature phantom. The results show a relative good agreement between both low-cost and high-end camera's and the phantom temperature gradient, with temperature differences of 1 degree increasing up to 3 degrees for high temperature (>80 C) between the camera and the phantom. The measurements were repeated as to absolute temperature and

temperature stability over the sensor area over a period of 1 hour. Both low-cost and high-end thermal cameras measured relative temperature changes with high accuracy and absolute temperatures with relative small variations. Low-cost smart phone based thermos cameras can be a good alternative to high-end thermos cameras for routine clinical measurements providing regular calibration checks for quality control.

10056-10, Session 3

Study on the origin to change physiochemical properties of polydimethylsiloxane phantoms for biomedical application

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Polydimethylsiloxane (PDMS) has been widely used in a variety of biomedical applications: microfluidic device, implant, and biomedical phantom due to its unique physiochemical and mechanical properties. To use PDMS properly, in-depth study for properties of PDMS is needed. Many studies for analysis of PDMS properties are suggested, however, there are shortages of systematic analysis and study on the origin of PDMS properties.

Typically, PDMS is produced by mixing and curing pre-polymer and curing agent with catalyst and thermal energy. The recommended mixing ratio (pre-polymer: curing agent) is typically 10:1 by raw materials suppliers. However, the reason why the mixing ratio 10:1 is considered proper than other mixing ratios has not been known clearly.

In this research, we presented the change of physicochemical properties of PDMS according to the mixing ratio and figured out the origin to change PDMS physiochemical properties by Raman spectroscopy and absorption spectroscopy.

We produced PDMS samples with various mixing ratios(1:1, 1.5:1, 2:1, 3:1, 5:1, 7:1, 9:1, 10:1, 12:1, 20:1, 30:1) and analyzed mechanical, optical, and surface properties of PDMS by measuring Young's modulus, OCT, hydrophobicity, and surface profiles according to the mixing ratio of PDMS. Also, we demonstrated the chemical composition of PDMS is changed when the mixing ratio of PDMS is changed by measuring Raman spectra and absorption spectra.

As a result, when the mixing ratio is about 9:1, the mechanical, optical, and surface properties of PDMS had extreme points and the reason was explained quantitatively with the data of Raman spectra and absorption spectra.

10056-11, Session 3

Simulating tissue scattering and birefringent properties in solid phantoms

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Polarization of biological tissue reflects birefringent characteristics of tissue components such as collagenous and elastic fibers. Polarimetry imaging techniques have been widely explored for disease diagnosis and therapeutic guidance. However, no traceable standard is available for calibration and validation of the polarimetry devices, partially due to the lack of reliable and stable tissue-simulating phantoms that simulate tissue birefringence properties. We propose a new method to fabricate tissue simulating phantoms that simulate tissue scattering and polarization characteristics.

The substrate of the phantoms are made of polydimethylsiloxane (PDMS). The PDMS material is mixed with sucrose to simulate optical rotation characteristics of chiral molecules in tissue. Titanum dioxide (TiO_2) particles are used to simulate organelle scattering properties of tissue. An electrostatic spinning method produces thin filaments with designated orientation and polarization characteristics to simulate collagen and elastic fiber orientation in biological tissue.By adjusting the concentration of the scattering particles and the arrangements of the fibers, the produced phantoms present different polarization characteristics. The proposed tissue-simulating phantoms can be potentially used to validate and calibrate the polarimetry medical devices.

10056-12, Session 3

Evaluation of a multi-layer diffuse reflectance spectroscopy system using optical phantoms

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We have previously developed a system combining diffuse reflectance spectroscopy (DRS, 475-850 nm) and laser Doppler flowmetry (LDF, 785 nm) with a model based analysis for absolute quantification of the skin microcirculation. The skin model consists of three layers, one bloodless (epidermis) layer and two dermal layers. The model is controlled by 11 parameters affecting the DRS spectra. They describe the thickness of the bloodless layer (1 parameter), the scattering properties (3 parameters), the melanin concentration and spectral shape (2 parameters), the blood tissue fraction (2 parameters), the oxygen saturation (2 parameters), and the average blood vessel diameter (1 parameter). Those 11 parameters are fitted to measured DRS spectra at two source-detector separations (0.4 and 1.2 mm).

Here we present results that characterize DRS parameters within the context of; 1) the effects of model parameters and 2) index matching fluid at the boundary between the probe and sample. The optical properties of the phantoms have been characterized and verified with independent optical techniques (diffuse optical spectroscopy/frequency domain photon migration and double integrating spheres and spatial frequency domain imaging). Layered phantoms consist of a thin epidermis simulating layer (100 – 300 um) and a thick layer simulating the dermis. Phantoms for the index matching investigation are based on epoxy phantoms (INO, Québec, Canada). Both forward and inverse methods have been employed.

The results show that forward Monte Carlo simulations for epoxy phantoms using index matching oil gives intensities within 10%, while without oil, simulated intensities have errors >20%.

10056-13, Session 4

Assessment of factors influencing infrared thermographic fever screening

Pejhman Ghassemi, T. Joshua Pfefer, Jon Casamento, Quanzeng Wang, U.S. Food and Drug Administration (United States)

Thermal modalities represent the only currently viable mass fever screening approach for outbreaks of infectious disease pandemics such as Ebola and SARS. Non-contact infrared thermometers (IRTMs) and thermographs (IRTGs) have been commonly used for mass fever screening in public areas such as airports. While IRTMs remain a more popular choice for fever screening, there has been increasing evidence in the literature that IRTGs can provide greater accuracy in estimating core body temperature if appropriate best practices are consistently applied.

Therefore, the purpose of this study was to develop a battery of evaluation test methods for standardized, objective and quantitative assessment of the influence of several factors on the use of IRTGs for temperature measurement at the inner canthi regions. These factors include device performance characteristics (e.g., spatial resolution, accuracy, stability) and other parameters that can be controlled to optimize device use (e.g., environmental conditions, subject orientation and distance). Two different commercial IRTG systems were examined. An external temperature reference source (a blackbody) with high temperature accuracy was utilized as part of the IRTG system. Results showed that both IRTGs are relatively accurate and stable (<1% error of reading with stability of ~±0.05˚C). A well-controlled temperature/humidity chamber was used to vary ambient conditions. Results showed a minimal change in temperature reading (<0.2%). Overall, results of this study may facilitate development of standardized consensus test methods to enable consistent and accurate use of IRTGs for fever screening.

10056-14, Session 4

Ray-traced Monte Carlo simulation tool for computer-aided design of tissue fluorescence sensing systems

Seung Yup Lee, Mary-Ann Mycek, Univ. of Michigan (United States)

In designing fluorescence spectroscopy and imaging systems for biological applications, a sample's own optical properties (scattering and absorption) can significantly affect system performance by altering the light path trajectory and attenuating its intensity. Monte Carlo (MC) simulation has been widely used to design fluorescence sensing systems by investigating those effects and reconstructing (optimizing) an intrinsic fluorescence signal. However, conventional MC codes have limitations in incorporating a variety of optical components, (e.g., graded-index and aspherical lenses, a series of different lenses) due to the complex mathematical equations for ray-tracing through those lenses.

Here, we describe a ray-traced Monte Carlo (RTMC) simulation tool to track a complete path of fluorescence excitation, propagation, and detection throughout the optical components and turbid media by combining MC codes and commercial ray-tracing software (ZEMAX®). ZEMAX calculates the ray path through an optical system including a source, detector, and optical components, and MC codes provide the photon trace inside the turbid media (tissue) sample.

Computational verification of RTMC was performed on reflectance and fluorescence measurements on layered tissue models in comparison with the gold-standard Monte Carlo code. RTMC was experimentally verified via depth-sensitive, steady-state fluorescence measurements using an aspherical lens on thin two-layered fluorescence phantoms.

The verification results demonstrate that the RTMC simulation has the potential to become a useful tool for designing tissue fluorescence imaging and spectroscopy systems. In addition, RMTC opens the door to a wide variety of applications for computational modeling of fluorescence in turbid media.

10056-15, Session 4

Near-infrared fluorescence image quality test methods for standardized performance evaluation

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Near-infrared fluorescence (NIRF) imaging has gained much attention as a clinical method for enhancing visualization of cancers, perfusion and biological structures in surgical applications where a fluorescent dye (e.g., Cy5, indocyanine green) is monitored by an imaging system. In order to address the emerging need for standardization of this innovative technology, it is necessary to develop and validate test methods suitable for objective, quantitative assessment of device performance. Towards this goal, we identify best practices and develop target-based test methods for key NIRF imaging system performance characteristics including spatial resolution, depth of field, uniformity, sensitivity and linearity. Characterization of fluorescence properties was performed by generating excitation-emission matrix properties of indocyanine green and quantum dots in biological solutions and matrix materials. A turbid, fluorophore-doped target was used to evaluate illumination/detection uniformity and – along with a USAF 1951 bar chart target to generate contrast transfer function for assessing spatial resolution. Multiwell plates filled with either liquid or were generated to explore best practices for evaluating detection linearity and lowest detectable concentrations of fluorophores. Finally, test methods were used to quantify the performance of CCD- and mobile-phone-based NIRF imaging systems as a function o+f illumination and detection parameters. Overall, our results demonstrate the utility of objective, quantitative, target-based testing approaches as well as the need to consider a wide range of factors in establishing standardized approaches for NIRF imaging system performance.

10056-16, Session 4

Traceable working standards with SI units of radiance for charactering the measurement performance of investigational clinical NIRF imaging devices

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All medical devices for Food and Drug market approval require specifications of performance based upon International System of Units (SI) or units derived from SI for reasons of traceability. Recently, near-infrared fluorescence (NIRF) imaging devices of a variety of designs have emerged on the market and in investigational clinical studies. Yet the design of devices used in the clinical studies vary widely, suggesting variable device performance. Device performance depends upon optimal excitation of NIRF imaging agents, rejection of backscattered excitation and ambient light, and selective collection of fluorescence emanating from the fluorophore. There remains no traceable working standards with SI units of radiance to enable prediction that a given molecular imaging agent can be detected in humans by a given NIRF imaging device. Furthermore, as technologies evolve and as NIRF imaging device components change, there remains no standardized means to track device improvements over time and establish clinical performance without involving clinical trials, often costly. In this study, we deployed a methodology to calibrate luminescent radiance of a stable, solid phantom in SI units of mW/cm^2/sr for characterizing the measurement performance of ICCD and IsCMOS camera based NIRF imaging devices, such as signal-to-noise ratio (SNR) and contrast. The vertical and horizontal resolutions of these NIRF camera systems were also assessed at the calibrated irradiance. The methodology allowed determination of superior SNR of the ICCD over the IsCMOS system; comparable contrast of ICCD and IsCMOS depending upon binning strategies; and superior resolution of the IsCMOS over the ICCD camera system.

10056-17, Session 4

Image quality assessment for teledermatology: from consumer devices to a dedicated medical device

Marine Amouroux, Univ. de Lorraine (France)

Aging population combined with growing incidence of type 2 diabetes induces a heavy prevalence of chronic skin disorders. In the meantime, chronic shortage of dermatologists leaves some areas underserved. Remote triage and assistance to homecare nurses (e.g. "teledermatology") appear to be promising solutions to provide patients living in remote areas with dermatological evaluation in an optimized time delay. Nowadays, teledermatology is often based on consumer devices (smartphones, digital cameras, tablets) whose photobiological and electrical features do not match medical devices' standards. The American Telemedicine Association (ATA) has published recommendations on quality standards for teledermatology. However, this "quick guide" does not address the issue of image quality which is critical in domestic environments where lighting (and many other parameters) is not reproducible. Standardized approaches of image quality would allow clinical trial comparison, calibration, manufacturing quality control and quality insurance during clinical use. Therefore, we developed a cost effective and portable device dedicated to teledermatology matching medical devices' safety requirements according to the 93/42/EEC Council Directive. Then several image features were selected in order to define image quality in teledermatology such as resolution, lightening uniformity, color repeatability and discrimination of key couples of colors on a color chart. Using such metrics, we compared quality of images produced by our device to images produced by consumer and medical devices that are not dedicated to teledermatology such as dermoscops. A clinical trial is scheduled in order to evaluate the impact of such a teledermatology - dedicated device on teledermatology consultations for retirement homes' residents.

10056-18, Session 4

Simulating tissue oxygenation by encapsulating hemoglobin in polymer microcapsules

Guangli Liu, Qiang Wu, Shu Wei Shen, Wenqi Ren, Peng Liu, Gang Zhao, Erbao Dong, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

We describe a combination of liquid-jet microencapsulation and molding techniques to fabricate tissue-simulating phantoms that mimick functional characteristics of tissue oxygen saturation (StO2). Chicken hemoglobin (Hb) was encapsulated inside a photocurable resin by a coaxial flow focusing process. The microdroplets were cured by ultraviolet (UV) illumination to form Hb loaded polymersome microdroplets. The microdroplets were further freeze-dried to form semipermeable solid microcapules with an outer transparent polymeric shell and an inner core of Hb. The diameter of the microcapsules ranged from 50 to100 ?m. The absorption spectrum of the microcapsules was measured by a UV/VIS spectrophotometer over a wavelength range from 400 nm to 1100 nm. To fabricate the tissue-simulating phantom, the Hb loaded microcapsules were dispersed in transparent polydimethylsiloxane (PDMS). The optical properties of the phantom were determined by an vertical double integrating sphere with a reconstruction algorithm. The experimental results showed that the tissue-simulating phantom exhibited the spectral characteristics closely resembling that of oxy-hemoglobin. The phantom had a long-term optical stability when stored in 4 ?, indicating that microencapsulation effectively protected Hb and improved its shelf time. With the Hb loaded microcapsules, we will produce skin-simulating phantoms for quantitative validation of multispectral imaging techniques. To the best of the authors' knowledge, no solid phantom is able to mimick living tissue oxygenation with good agreement. Therefore, our work provided an engineering platform for validating and calibrating spectral optical devices in biomedical applications.

10056-19, Session 5

Depth-of-focus extended chromatic dual-foci OCT

Jinhan Li, Nanyang Technological Univ. (Singapore)

The depth scanning range of high-resolution OCT is limited by its depth-of-focus (DOF). To solve this problem, we developed a chromatic dual-foci technology to maintain a transverse resolution of 1-2 um over a DOF of about 300 micrometers, which is 2-3 times larger than the single-focus OCT system. In this OCT system, a supercontinuum source is used to provide illumination from 700 to 1600 nm. The interference fringe is detected by a dual-spectrometer system, which engages a Si camera and an InGaAs camera. The Si camera detects the spectral single from 700 to 950 nm, and InGaAs camera covers 1100 to 1600 nm spectral range. As the focal region for long wavelength section is deeper than the short one, the two spectral sections are processed separately to form two OCT images of different depths in the sample. After combining the two images, we obtained DOF extended OCT images.

10056-20, Session 5

A comparative study of noise in supercontinuum light sources for ultra-high resolution optical coherence tomography at 1300 nm

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Supercontinuum (SC) light is a well-established technology, which finds applications in several domains ranging from chemistry to material science and imaging systems [1-2]. More specifically, its ultra-wide optical bandwidth and high average power make it an ideal tool for Optical Coherence Tomography (OCT). Over the last 5 years, numerous examples have demonstrated its high potential [3-4] in this context. However, SC light sources present pulse-to-pulse intensity variation that can limit the performance of any OCT system [5] by degrading its signal to noise ratio (SNR). To this goal, we have studied and compared the noise of several SC light sources and evaluated how their noise properties affect the performance of Ultra-High Resolution OCT (UHR-OCT) at 1300 nm. We have measured several SC light sources with different parameters (pulse length, energy, seed repetition rate, non-linear fiber characteristics etc).

In addition to the source comparison, we have also studied the optimal interfacing of a SC source with an UHR-OCT setup. We have investigated how the SC seed power, camera exposure time or signal power level in the system affect the SNR. We show that it is possible to identify operation regimes where the shot noise dominates, hence providing an optimal working point for the system with up to 10 dB improvement in the SNR.

We illustrate the different noise measurements and their impact on a state of the art UHR-OCT system producing in-vivo images of skin. The sensitivity of the system was higher than 95 dB, with an axial resolution below 4μm.

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10056-21, Session 5

Analysis of polygonal scanning heads: from industrial to high-end applications in swept sources for OCT

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An analysis of polygonal mirror (PM) scanning heads is performed, in order to provide a tool for their optimal design. The theory developed brings uder the same umbrella applications that range from industrial dimensional measurements to high-end biomedical imaging, for example for broadband laser sources swept in frequency for Swept Source Optical Coherence Tomography (SS-OCT). The different characteristic parameters of the PM scanning heads are considered in order to achieve a rigorous analysis: number of PM facets, inner radius of the PM, eccentricity of the PM pivot with regard to the incident fixed laser beam, distance from this beam to the principal plane of the objective lens, and angular velocity of the PM. The functions that characterize the PM scanning head are deduced: scanning function and velocity, characteristic angles, duty cycle, and two migration functions (longitudinal and transversal) that allow for an optimized designing calculus of the PM placed in different SS configurations. The analytic and numerical analysis of these functions is performed with regard to the characteristic parameters of the scanning head. Experimental validations of the theory are completed. While the above analysis is carried out for a characteristic ray of the collimated laser beam (i.e., its central axes), it also allows for considering in a simple way the finite diameter of the beam. Thus, a discussion on the deformation of the beam – as produced by the eccentric rotational PM facet - concludes the study. A comparison of the PM scanner with the most common types of laser scanners highlights its advantages and drawbacks for OCT. Selected Refs.: Duma V.-F., Polygonal mirror laser scanning heads: Characteristic functions, Proc. of the Romanian Academy A 18(1), (2017) – accepted; Duma V.-F.*, Tankam P., Huang J., Won J. J., and Rolland J. P., Optimization of galvanometer scanning for Optical Coherence Tomography, Appl. Opt. 54(17), 5495-5507 (2015); Duma V.-F.*, Lee K.-S., Meemon P., Rolland J. P., Experimental investigations of the scanning functions of galvanometer-based scanners with applications in OCT, Appl. Opt. 50(29), 5735-5749 (2011). Duma V.-F., Optimal scanning function of a galvanometer scanner for an increased duty cycle, Opt. Eng. 49(10), 103001 (2010).

10056-22, Session 5

Integrated-optics based multi-beam imaging for speed improvement of OCT systems

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The frame rate of an optical coherence tomography (OCT) system is limited by the speed of the camera or the sweep rate of the light source. This problem can be overcome by multiple-beam imaging, in which different locations on the sample are illuminated by an array of light simultaneously. This technique allows parallel imaging from multiple sample locations and therefore improves OCT axial scan rate by a factor equal to the number of beams used simultaneously which can go up to very high frequency ranges (e.g. MHz) easily. We proposed a very compact integrated-optics based multiple-beam illumination design in which several wavelength-independent couplers are concatenated in series by using optical waveguides with certain delay lines. There will be electrodes on top of the connecting waveguides between primary and the secondary couplers to make the design reconfigurable. The other benefit of using these electrodes is the ease of separating desired signal from unwanted reflections at the optical surfaces or tissue. In total there will be N optical waveguides followed by N on-chip focusing lenses. The number of waveguides will provide N times speed improvement in the imaging. In addition to fast imaging, the proposed design will be very compact, cheap, and robust. The speed improvement is the game-changer in OCT-imaging cause it opens up for new and very exciting applications.

10056-42, Session 6

Open explorations of the microcosmos

Manu Prakash, Stanford Univ. (United States)

Using principles of "frugal science" - I will discuss a few ideas from our lab where we are imagining how to enable open explorers (traditional and non-traditional scientists) around the world to ask and discover curiosity driven questions in biological, ecological and health related domains. More specifically I will describe the history and making of "Foldscope", an ultra-low cost origami based print-and-fold paper microscope that brings microscopy out of the lab and into the hands of kids and adults globally. Manufactured with a roll-to-roll printed optics that applies paper folding to make optical instruments, Foldscope is a field microscope that enables a broad range of biological explorations in field conditions. Our long term vision is to bring "microscopy to every child in the world"; and enable a curiosity driven approach to exploring biological mysteries at an early age and encourage explorations of the micro-cosmos.

10056-24, Session 7

Surgical instrument biocontaminant fluorescence detection in ambient lighting conditions for hospital reprocessing and sterilization department

François Baribeau, Annie Bubel, Guillaume Dumont, Carl Vachon, André Lépine, Stéphane Rochefort, Martin Massicotte, Louis Buteau-Vaillancourt, Pascal Gallant, Ozzy Mermut, INO (Canada)

Hospitals currently rely on simple human visual inspection for assessing cleanliness of surgical instruments. Studies showed that surgical site infections are in part attributed to inadequate cleaning of medical devices. Standards groups recognize the need to objectively quantify the amount of residues on surgical instruments and establish guidelines. We developed a portable technology for the detection of contaminants on surgical instruments through fluorescence following cleaning. Weak fluorescence signals are usually detected in the obscurity only with the lighting of the excitation source. The key element of this system is that it works in ambient lighting conditions, a requirement to not disturb the normal workflow of hospital reprocessing facilities. A biocompatible fluorescent dye is added to the detergent and labels the proteins of organic residues. It is resistant to the harsh environment in a washer-disinfector. Two inspection devices have been developed with a 488nm laser as the excitation source: a handheld scanner and a tabletop station using spectral-domain and time-

domain ambient light cancellation schemes. The systems are eye safe and equipped with image processing and interfacing software to provide visual or audible warnings to the operator based on a set of adjustable signal thresholds. Micron-scale residues are detected by the system which can also evaluate soil size and mass. Unlike swabbing, it can inspect whole tools in real-time. The technology has been validated in an independent hospital decontamination research laboratory. It also has potential applications in the forensics, agro-food, and space fields. Technical aspects and results will be presented and discussed.

10056-25, Session 7

Laser assisted robotic surgery in cornea transplantation

Francesca Rossi, Filippo Micheletti, Istituto di Fisica Applicata “Nello Carrara” (Italy); Giada Magni, Istituto di Fisica Applicata (Italy); Roberto Pini, Istituto di Fisica Applicata “Nello Carrara” (Italy); Luca Menabuoni M.D., Azienda USL 4 (Italy); Fabio Leoni, Scuola Superiore Sant’Anna (Italy) and Fastenica srl (Italy); Bernardo Magnani, Ekymed srl (Italy)

Robotic surgery is a reality in several surgical fields, such as in gastrointestinal surgery. In ophthalmic surgery the required high spatial precision is limiting the application of robotic system, and even if several attempts have been designed in the last 10 years, only some application in retinal surgery were tested in animal models. The combination of photonics and robotics can really open new frontiers in minimally invasive surgery, improving the precision, reducing tremor, amplifying scale of motion, and automating the procedure. In this manuscript we present the preliminary results in developing a vision guided robotic platform for laser-assisted anterior eye surgery. The robotic consolle is composed by a robotic arm equipped with an “end effector” designed to deliver laser light to the anterior corneal surface. The main intended application is for laser welding of corneal tissue in laser assisted penetrating keratoplasty and endothelial keratoplasty. The consolle is equipped with an integrated vision system. The experiment originates from a clear medical demand in order to improve the efficacy of at least 20 different surgical procedures: when the prototype will be optimized, other surgical areas will be included in its application, such as neurosurgery, urology and spinal surgery. This research activity has been performed in the framework of the Experiment “LA-ROSES”, granted by FP7 ECORD++ - The European Coordination Hub for Open Robotics Development (2015-2016).

10056-26, Session 7

Design and implementation of a dual-wavelength intrinsic fluorescence camera system

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Intrinsic UV fluorescence imaging is a technique that permits the observation of the spatial differences in emitted fluorescence. It relies on the fluorescence produced by the innate fluorophores in the sample, and thus can be used for marker-less in-vivo assessment of tissue. It has been studied as a tool for the study of the skin, specifically for the classification of lesions, the delimitation of lesion borders and the study of wound healing, among others. In its most basic setup, a sample is excited with a narrow-band UV light source and the resulting fluorescence is imaged with a UV sensitive camera filtered to the emission wavelength of interest. By carefully selecting the excitation/emission pair, we can observe changes in fluorescence associated with physiological processes. One of the main drawbacks of this simple setup is the inability to observe more than a single excitation/emission pair at the same time, as some phenomena are better studied when two or more different pairs are studied simultaneously. In this work, we describe the design and the hardware and software implementation of a dual wavelength portable UV fluorescence imaging system. Its main components are an UV camera, a dual wavelength UV LED illuminator (295 and 345 nm) and two different emission filters (345 and 390 nm) that can be swapped by a mechanical device. The system is operated using a laptop computer and custom software that performs basic processing to improve the image. The system was designed to allow us to image fluorescent peaks of tryptophan and collagen cross links in order to study wound healing progression.

10056-27, Session 7

On the origin of the visible light responsible for proton dose measurement using plastic optical fibers

Arash Darafsheh, Perelman Ctr. for Advanced Medicine, Univ. of Pennsylvania (United States); Reza Taleei, Univ. of Texas SW Medical Ctr. (United States); Alireza Kassaee, Jarod C. Finlay, Univ. of Pennsylvania (United States)

We studied the origin of the visible light responsible for proton dose measurement using plastic optical fibers. Our spectroscopic study results, validated by Monte Carlo simulation, showed that ?erenkov radiation is not the responsible signal for proton dose measurement using optical fibers.

10056-28, Session 7

Reliable determination of tissue optical properties from spatially resolved reflectance

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Spatially resolved reflectance is a frequently used technique to derive optical properties and physiological parameters of tissue. We evaluate the accuracy of this method for source-detector distances covering a range from about 1 mm up to typically 10 mm or even 20 mm which enable us to look several millimeters deep into the scattering medium. Experimentally, we employ either a free-space light detection setup or a linear fiber probe. Measurements have been carried out on various phantoms with known optical properties derived from independently performed time-resolved measurements. Spatially resolved reflectance was analyzed by a Monte Carlo model as well as by diffusion theory for comparison. With the Monte Carlo model we also take the effects of the detection aperture into account. To evaluate the optical properties retrieved by curve fitting, we use systematic investigations of the dependency of the chi square

minimization function on the optical properties. Without any calibration of the reflectance data the minimum is typically flat which results in a poor estimation of the true optical properties together with very large error bars. The separation of scattering and absorption coefficients is strongly improved when the reflectance data are calibrated. We apply our methods to in vivo investigations on renal tissue in a small animal model in which a limited number of detection fibers can be used only. Based on the estimated optical properties hemoglobin concentration and Hb oxygen saturation are quantified using the calibration approach, and uncertainties of these data are estimated.

10056-23, Session PSun

Single camera full range high resolution spectral domain optical coherence tomography

En Bo, Linbo Liu, Nanyang Technological Univ. (Singapore)

We developed a spectral domain optical coherence tomography (SD-OCT) using a 3?3 fused-fiber coupler for complex conjugate artifacts (CCA) suppression. Two real interferometric spectra sharing the same spectrometer optics were obtained simultaneously by two lines of a three-line charge coupled device (CCD). The complex interferometric signal was reconstructed by trigonometric manipulation of two real interferometric spectra, and then inverse Fourier transform was performed to obtain full-range imaging. However, the remaining artifacts at direct current (DC) and a ghost remnant of the CCA in the resolved A-scan were observed due to mismatching in the spectrometers. To address such an issue, careful matching of two spectrometers was required such that the exact same wavelength range was sampled in each corresponding pixel of the two lines of the three-line CCD, since slight mismatching of the spectrometers had strong negative effects on CCA suppression and appeared to be the limiting factor on system performance. This novel spectrometer with three-line CCD helped achieve a better matching of two spectrometers, and consequently better performance in CCA suppression as compared to the dual spectrometer design. Full-range imaging with ~25dB suppression of CCA was demonstrated when imaging an attenuated reflector. The efficacy of both CCA and DC suppressions was also validated by imaging the anterior segment of a rat eye ex vivo. The quality of CCA-suppressed images was significantly improved with regard to those obtained with the dual-spectrometer design.

10056-30, Session PSun

Scanning-free multimode fiber based endoscope

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Previously demonstrated endoscopes working in reflection mode necessitate a certain form of scanning (e.g., plane wave, sharp spot and random pattern). In this work, however, we attempt to demonstrate a scanning-free single multimode fiber (MMF) based endoscope by theoretical modeling. With a spatial light modulator (SLM) and the pre-measured transmission matrix (TM) from calibration, a controlled illumination field (e.g., uniform illumination) can be generated through the imaging MMF. The captured single image is then recovered by TM method before the illumination impact is filtered out. This allows the direct acquisitions of the original image in one go, thereby eliminating the need of scanning at the proximal end. Such workflow leads to a truly scanning-free and single-optical-fiber endoscope. In this work, recovery abilities of different multimode fibers are studied at first in order to generate accurately controlled illumination fields. It is found that supported mode number should be over image pixel amount to achieve satisfactory recovery results. Speaking of our instance, correlation coefficient can be over 0.98 at the turning point. Onward, scanning-free endoscope is demonstrated for uniform illumination as well as even input. Note that our method is valid for arbitrary controlled illumination fields though. For both illumination conditions, correlation coefficients over 0.99 are obtained. But for the latter, additional illumination impact filtering is necessary since object is not illuminated evenly. At last, the proposed scanning-free approach is compared with scanning methods in terms of imaging quality. It is shown that single-frame approach can greatly improve imaging efficiency while keeping image quality well.

10056-31, Session PSun

Development of an antimicrobial blended white LED system containing pulsed 405-nm LEDs for decontamination applications

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Optical disinfection technologies, along with other non-antibiotic based-strategies, are becoming more prominent in the fight against healthcare-associated infection. 405-nm light has significant antimicrobial activity, and although less germicidal than ultraviolet light, has the advantage that it can be used at levels which will not harm mammalian cells. These benefits have permitted the development of 405-nm light for continuous environmental decontamination, and this study investigates the potential operational advantages of using pulsed 405-nm light-emitting diodes (LEDs) for this antimicrobial application.

Initial proof-of-concept tests compared the optical efficiency, energy consumption and antimicrobial efficacy of pulsed versus continuous light. The parameters of the pulsed system (duty cycle, frequency, peak intensity and dose) were varied and 99.9% reduction in the populations of Staphylococcus aureus were achieved, with results showing >80% increase in optical efficiency, and reduced energy consumption under pulsed conditions.

The study then progressed to investigate the pulsed operation of 405-nm, red, yellow and green LEDs to develop an antimicrobial, blended white light system with improved aesthetic output compared to existing 405-nm decontamination systems, blended with white LEDs. This blended system was developed using pulse width modulation to control the intensity of each of the LEDs, and a lens-diffuser setup for enhanced colour blending. Results demonstrating the optical efficiency, antimicrobial efficacy and improved photometric output of this system will be presented.

Overall, this study concludes that pulsed 405-nm LEDs can have operational advantages over continuous light for infection control, decontamination applications, including increased optical efficiency and improved aesthetic output, and warrants further investigation.

10056-33, Session PSun

Multispectral fluorescence diffuse optical tomography

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Fluorescence diffuse optical tomography (FDOT) has been widely used for in vivo small animal studies and the ill-posed problem in reconstruction

can be eased by utilizing structural a priori obtained from an anatomic imaging modality. In this study, a multispectral fluorescence tomography (FT) is used, which has shown the ability to detect subtle shifts in the ICG absorption spectrum in our previous study. The imaging system is in trans-illumination mode with a swept-wavelength laser and a CCD on a rotation gantry and the structural image from the X-ray computed tomography is used to guide and constrain the FT reconstruction algorithm. In this work, a phantom with two inclusions filled with different fluorophores is utilized to evaluate whether the spectral information obtained using swept-wavelength laser can distinguish these two inclusions. The images are captured from 8 different views with three different wavelengths.

10056-34, Session PSun

3D splint prototype system for applications in Muscular Rehab by Transcutaneous electrical nerve stimulation (TENS)

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We are proposing a prototype for medical applications that reduces the cost and accelerates the rehabilitation process in patients that has some issues with the upper limbs such as ligamentous rupture, tendinitis, tenosynovitis, sprains among other kind of injuries that require the use of a splint. The prototype features a 3D scanner that develops a digital model through a CAD software co-integrated with a mechanical gear that fits properly the patient upper limb. Once scanned and analyzed, the splint is printed among other materials, SLA. In order to improve the patient recovery time, a device was integrated into the splint design that works as a rehabilitator by using a set of electrical impulses according to TENS. This device will be either added or activated according to the best judgment of the GP. The frequency will be applied through an array of graphene electrodes due to its particular electrical and mechanical properties.

10056-35, Session PSun

Development of a hybrid NIRS/LDF sensor system for microcirculation detection

Ting-Ying Li, Chia-Wei Sun, National Chiao Tung Univ. (Taiwan)

Microcirculation presents in the small vasculature embedded within tissue and it deals with the circulation of blood from the heart to arteries, veins and capillaries. The microcirculation could response the physiological condition of human.

Near-infra-red spectroscopy (NIRS) can provide non-invasive and quantitative measurements of haemoglobin concentration and oxygenation. Laser Doppler flowmetry (LDF) provides estimates of local blood perfusion in superficial tissues for, e.g. skin. In this study, we built a hybrid near-infrared / laser Doppler flowmetry system for microcirculation detection. The near-infrared diffuse photon penetrates deeper tissue and reveals the information of microcirculation. We propose the near-infrared illumination as a non-invasive physiological intervention for laser Doppler measurement. The optical assessment shows an interpretation of tissue microcirculation change with oxygenation and blood perfusion dynamics.

10056-36, Session PSun

Continuous control systems for non-contact ECG

Vladimir L. Kodkin, Alexey Smirnov, Galina V. Yakovleva, South Ural State Univ. (Russian Federation)

South Ural State University is still conducting studies dedicated to development of complex systems designed for functional diagnosis of the human state and based on non-contact ECG recording. The scientists developed several devices making it possible to obtain a precise ECG without using special items with belts, gel etc.

High-accuracy software-hardware systems allowed the researchers to acquire almost continuous ECG control by means of conducting fabrics and threads “embedded” in clothes. The signal quality is high enough, so it is possible not only calculate heart rate variability as in many similar systems, but also to record separate ECG elements such as peaks and intervals, at the level of 70-100 μV. These advantages may be important if the system is used in obstetrics for a pregnant woman’s abdominal ECG and fetal ECG distinguishing as fetal ECG amplitude in abdominal ECG is 10-100 μV and changes very quickly.

Based on the conducted studies the prototype continuous control systems for ECG are being developed.

10056-37, Session PSun

A capillary-mimicking optical tissue phantom for diffuse correlation spectroscopy

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Optical tissue phantoms are necessary for instrument benchmarking and providing a consistent baseline for experiments in various fields of tissue spectroscopy, including diffuse correlation spectroscopy (DCS). To provide the most useful comparisons, a phantom would ideally mimic tissue as closely as possible, including the geometry of static and dynamic scatterers. Human capillaries have diameters (~100 microns) that are difficult to replicate with existing 3D printing technology. We demonstrate a 3D printed phantom that is within one order of magnitude of this critical dimension, while previous work could only reach factors of 100-1000. A branching design that keeps the flow cross section constant ensures that the same flow velocity is found throughout the phantom while allowing for single input and output fittings to feed all of the capillaries simultaneously. The direction of each capillary is randomized every few millimeters by randomly allocating 2x2 “twisting” squares within each layer. These squares swap the locations of four adjacent artificial capillaries either clockwise or counter-clockwise. Numerical simulations were used to verify the random walk-like behavior of the capillary paths resulting from this pattern. This is a step toward replicating the randomly varying directionality of actual capillaries. This design was verified by taking DCS measurements at different flow rates of Intralipid through the phantom, demonstrating the dependence of the exponential decay of the autocorrelation on the flow rate.

10056-38, Session PSun

Towards a clinical implementation of micro-optical coherence tomography instrument for in vivo imaging of human airway

Hui Min Leung, Wellman Ctr. for Photomedicine (United States) and Massachusetts General Hospital; Harvard Medical School (United States); Dongyao Cui M.D., Nanyang Technological Univ. (Singapore) and The Wellman Ctr. for Photomedicine (United States) and Massachusetts General Hospital (United States); Kengyeh K. Chu, Duke Univ. (United States); Guillermo J. Tearney M.D., Wellman Ctr. for Photomedicine (United States) and Massachusetts General Hospital, Harvard Medical School (United States)

High resolution micro-optical coherence tomography (μOCT) technology has been demonstrated to be useful for imaging respiratory epithelial functional microanatomy relevant to the study of pulmonary diseases such as cystic fibrosis and COPD. Based on the principles of spectral-domain OCT, the instrument is capable of real-time imaging at 40 fps at lateral and axial resolutions of 2 and 1.3μm, respectively. A benchtop implementation of the imaging technology has allowed several relevant parameters to be quantified in vitro, including airway surface liquid, ciliary beat frequency and the rate of mucociliary transport. To translate this technology to evaluate human airways in vivo, we are developing a portable μOCT imaging system with comparable optical performance. The system consists of two main subsystems, a flexible μOCT endobronchial probe and a portable imaging console. The fiber-based probe is a common-path interferometer designed to be compatible with a standard bronchoscope. It features an annular apodization of the sample arm beam to achieve an extended depth of focus. A high-power (~6W) supercontinuum laser source is utilized and the interferogram returning from the probe is spectrally dispersed and recorded on a CCD. The spectrometer, which is built in-house and installed on a portable cart, comprises a 900 lpmm holographic grating and a 4095-pixel line scan camera. Multiple features are incorporated into the imaging system to ensure safe human use of this high power laser source in a clinical setting. In addition to the development of the portable system, preliminary imaging results are shown to demonstrate the feasible clinical use of the μOCT technology.

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10057-1, Session 1

Speckle reduction using two step iteration in optical coherence tomography

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Optical coherence tomography (OCT) provides high resolution and cross-sectional images of biological tissue and is widely used for diagnosis of ocular diseases. However, OCT images suffer from speckle noise, which typically considered as multiplicative noise in nature, reducing the image resolution and contrast. In this study, we propose a two-step iteration (TSI) method to suppress those noises. We first utilize augmented Lagrange method to recover a low-rank OCT image and remove additive Gaussian noise, and then employ the simple and efficient split Bregman method to solve the Total-Variation Denoising model. We validated such proposed method using images of swine, rabbit and human retina. Results demonstrate that our TSI method outperforms the other popular methods in achieving higher peak signal-to-noise ratio (PSNR) and structure similarity (SSIM) while preserving important structural details, such as tiny capillaries and thin layers in retinal OCT images. In addition, the results of our TSI method show clearer boundaries and maintains high image contrast, which facilitates better image interpretations and analyses.

10057-2, Session 1

Optical skin assessment based on spectral reflectance estimation and Monte Carlo simulation

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Optical non-contact measurements in general and chromophore concentration estimation in particular have been identified to be useful tools for skin assessment. Spectral estimation using a low cost hand held device has not been studied adequately as a basis for skin assessment. Spectral measurements on the one hand which require bulky, expensive and complex devices and simplified direct channel approaches on the other hand which operate with simple optical devices have been considered and applied for skin assessment. In this study we analyse the capabilities of spectral estimation for skin assessment in form of chromophore concentration estimation using a prototypical low cost optical non-contact device. A spectral estimation workflow is implemented and combined with pre-simulated Monte Carlo spectra to use estimated spectra for chromophore concentrations estimation and obtain health metrics. To evaluate the proposed approach we performed a series of occlusion experiments and examined the capabilities of the proposed process. Additionally the method has been applied to more general skin assessment tasks. Results obtained are both compared to chromophore estimations based on spectral measurements and to direct channel approaches. The proposed process provides a more general representation in form of a spectral image cube which can be used for more advanced analysis and the comparisons show good agreement with expectations and conventional skin assessment methods. Utilising spectral estimation in conjunction with Monte Carlo simulation could lead to low cost, easy to use, hand held and multifunctional optical skin assessment with the possibility to improve skin assessment and the diagnosis of diseases.

10057-3, Session 1

Fiber based fast sparse sampling X-ray luminescence computed tomography

Wei Zhang, Michael C. Lun, Changqing Li, Univ. of California, Merced (United States)

Super fine collimated x-ray beam based x-ray luminescence computed tomography (XLCT) has the potentials to recover the deeply embedded targets with a spatial resolution of hundreds of micrometers. However, due to the low x-ray photon utilization efficiency and low optical signal sensitivity of the electron multiplying charge coupled device (EMCCD) camera, the XLCT usually requires a long measurement time. To overcome this limitation, we propose a fiber based, fast XLCT design, in which optical fiber bundles are placed circularly to collect the emitted optical photons on the phantom surface. Highly sensitive photomultiplier tubes (PMT) with a cooling unit and pre-amplifier are used to measure the photons from the fiber bundles. The PMT outputs are collected by a high-speed data acquisition board. A linear scan is estimated to take about 5 seconds, thus for an XLCT scan with 6 projections, we require 30 seconds for each section, which makes it possible to have a whole body scan of XLCT. To validate our design, numerical simulations and phantom experiments have been performed. In numerical simulation studies, we have investigated the effects of the number of optical fiber bundle and angular projections on the XLCT reconstruction. We found that 4 optical fiber bundles are sufficient to reconstruct the deeply embedded targets if measurements of 6 projections are used. Phantom experiments with multiple targets will be performed in coming months to validate the proposed fast XLCT imaging. If successful, the proposed approach will make XLCT more practical for in vivo mice imaging.

10057-4, Session 1

Investigation of burn effect on skin using simultaneous Brillouin, Raman, and fluorescence microspectroscopy

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Burns are thermal injuries that can completely damage or at least compromise the protective function of skin, and affect the ability of tissues to manage moisture. Burn-damaged tissues exhibit lower elasticity than healthy tissues, due to significantly reduced water concentrations and plasma retention. Current methods for determining burn intensity are limited to visual inspection, and potential hospital x-ray examination. We present a unique confocal microscope capable of measuring Raman and Brillouin spectra simultaneously, with concurrent autofluorescence detection from a single spatial location, and demonstrate application by investigating and characterizing the properties of burn-afflicted tissue. Raman and Brillouin scattering offer complementary information about a material's chemical and mechanical structure, while autofluorescence can serve as a useful diagnostic indicator and imaging tool. The developed instrument has the potential for very diverse analytical applications in basic biomedical science and biomedical diagnostics and imaging.

10057-5, Session 2

Combined optical coherence tomography and hyper-spectral imaging

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Optical coherence tomography (OCT) is a powerful technique to evaluate tissue structure in many fields of medicine such as ophthalmology, gastroenterology and laryngology amongst many others. However, OCT provides only little insight into the composition of the sample. Hyper-spectral imaging (HSI) can address this shortcoming as the method yields 2D spectral information, which can be used to assess a sample's molecular content. Combining these modalities into a single tool is therefore clinically valuable as the complementary datasets will assist clinicians in their analyses and diagnostics.

In this work, we demonstrate the combination of OCT and HSI into a single double-clad fiber (DCF). The combination of two modalities into a single fiber is particularly interesting as it promotes the translation of the technology into miniaturized endoscopic tools for non- or minimally invasive procedures. Furthermore, both illumination and collection of the backscattered signal are carried out through the single DCF, removing the need for external illumination and thus further increasing the potential for miniaturization.

In the presented set-up, illumination for both modalities is performed through the fiber core. The backscattered signal is then collected in the core for OCT, and in the DCF inner-cladding for HSI. A double-clad fiber coupler (DCFC) is used to address both channels independently. In order to ensure rapid imaging as well as video-rate display for both modalities, we developed custom hardware for the acquisition of spectral data as well as custom software for simultaneous signal acquisition and processing of both modalities. Results are demonstrated in vivo.

10057-6, Session 2

Multimodal fluorescence and photoacoustic microscopy in the frequency domain

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We demonstrate multimodal optical-resolution frequency domain fluorescence and photoacoustic scanning microscopy with sub-μm lateral resolution. The fluorescence and photoacoustic signals are generated in chromophores by using a diode laser which is sinusoidally modulated in the MHz regime. The excitation light is focused to the sample via an objective lens. Fluorescence is collected by the same objective in a confocal configuration and measured by an avalanche photo diode. Photoacoustic waves are recorded from the opposite side of the sample using an ultrasonic transducer. Both, the fluorescence and the photoacoustic signals are simultaneously recorded using a lock-in technique. We demonstrate that photoacoustic and fluorescence images provide complementary information. In case a substance does absorb the excitation laser light, it does preferably generate photoacoustic signals if non-radiative recombination dominates. In case radiative recombination dominates the chromophore mainly shows luminescence. We present images of blood smears, fluorescent dyes, and stained cells cultures. The complementary information allows the discrimination between different chemical compositions.

In the case of pulsed excitation one can obtain the fluorescence life time by measuring the exponential decay rate of the fluorescence signal. The photoacoustic signal, however, does not provide information on the non-radiative relaxation times. In frequency domain both relaxation times can be determined by sweeping the modulation frequency of the excitation laser and investigation of, e.g., the phase information. Finally, we discuss the different physical mechanisms behind fluorescence and photoacoustics, leading to different behaviors concerning, e.g., the signal-to-noise ratios as a function of the excitation frequency.

10057-7, Session 2

A motorized endoscope for simultaneous optical coherence tomography and near-infrared fluorescence imaging

Fabio Feroldi, Helene Knaus, Johannes F. de Boer, Vrije Univ. Amsterdam (Netherlands)

Lung cancer is the most fatal form of cancer in the Western world. Addressing the type of cancer in an early stage is fundamental to increase the chances of treatment. Current standard techniques such as X-ray computed tomography and positron emission tomography are unable to resolve lesions smaller than a few millimeters. Optical techniques are suitable candidates to assess tumors as small as tens of micrometers. Optical coherence tomography (OCT) can provide three-dimensional structural images with a resolution of about 10 microns, but it lacks functional information. Targeted near-infrared fluorescence imaging (NIRF) is, on the other hand, able to accurately detect the presence of a particular fluorophore in a positively labeled tissue but lacks morphological information. The combination of these two techniques in a single device provides the opportunity for in-vivo assessment of tumors at an early stage. A motorized endoscope featuring double-clad optical fibers was developed to deliver the light at the location of interest and therefore simultaneously perform high-resolution OCT and efficiently recover the scattered fluorescent emission. A virtually all-fiber optical system was built featuring a swept source based OCT setup at 1310nm and a single photon fluorescence setup at 780nm. The device has been tested on phantoms, demonstrating the ability to detect concentrations of the fluorophore IR-dye800CW as small as 100nM, with an excitation power of 1.4mW. We also addressed technical issues such as Raman scattering in silica fibers.

10057-8, Session 2

Spectral band optimisation for multispectral fluorescence imaging

Dale J. Waterhouse, Siri A. Luthman, Sarah E. Bohndiek, Univ. of Cambridge (United Kingdom)

Multispectral imaging has the potential to improve sensitivity and specificity in biomedical imaging through simultaneous acquisition of both morphological (spatial) and chemical (spectral) information. Performing multispectral imaging in real time, for example in endoscopy or intraoperative imaging, requires a direct trade off between spatial and spectral resolution. We sought to quantitatively assess the impact of spectral band selection, including both center wavelength and bandwidth, on fluorescent contrast agent detection in molecular endoscopic imaging.

As a proof of concept, we used a 2:1 dilution series of a single fluorescent contrast agent (AlexaFluor647) and acquired 'ground truth' spectra using a spectrometer (AvaSpec-ULS2048, Avantes) incorporated into the detection path of our endoscope (Polyscope, Polydiagnost). We then modeled the influence of a filter-based multispectral imaging camera on these spectra and calculated the theoretical endmembers associated with reflectance and fluorescence signals from the pure contrast agent. To test the accuracy of our model, we incorporated into the same endoscope an off-the-shelf

multispectral CMOS sensor (CMS-V, SILIOS) with a 3x3 filter deposition pattern of 9 spectral bands.

After spectral unmixing using the modeled endmembers, the amplitude of the fluorescence recorded with the multispectral CMOS sensor compared favorably with the amplitude of fluorescence derived from the 'ground truth' spectra recorded with the spectrometer (R^2 = 0.99). In the future, this approach could be used to minimize the number of spectral bands required in a given imaging system and hence maximize the spatial resolution of the multispectral camera.

10057-9, Session 2

Multi-modal approach using Raman spectroscopy and digital holographic microscopy for the identification of immune cell subsets

Naomi McReynolds, School of Physics & Astronomy, Univ. of St. Andrews (United Kingdom); Fiona G. M. Cooke, School of Medicine, Univ. of St. Andrews (United Kingdom); Mingzhou Chen, School of Physics & Astronomy, Univ. of St. Andrews (United Kingdom); Simon J. Powis, School of Medicine, Univ. of St. Andrews (United Kingdom); Kishan Dholakia, School of Physics & Astronomy, Univ. of St. Andrews (United Kingdom)

Moving towards label-free techniques for cell identification is essential for many clinical and research applications. Raman spectroscopy and digital holographic microscopy (DHM) are both label-free, non-destructive optical techniques capable of providing complimentary information. We demonstrate a multi-modal system which may simultaneously take Raman spectra and DHM images to provide both a molecular and a morphological description of our sample. In this study we use Raman spectroscopy and DHM to discriminate between three immune cell populations CD4+ T cells, B cells, and monocytes, which together comprise key functional immune cell subsets in immune responses to invading pathogens. Various parameters that may be used to describe the phase images are also examined such as pixel value histograms or texture analysis. Using our system it is possible to consider each technique individually or in combination. Principal component analysis is used on the data set to discriminate between cell types and leave-one-out cross-validation is used to estimate the efficiency of our method. Raman spectroscopy provides specific chemical information but requires relatively long acquisition times, combining this with a faster modality such as DHM could help achieve faster throughput rates. The combination of these two complimentary optical techniques provides a wealth of information for cell characterisation which is a step towards achieving label free technology for the identification of human immune cells.

10057-10, Session 2

Dual-modality smartphone endoscope for cervical pre-cancer detection

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Early detection is the key to the prevention of cervical cancer. There is an urgent need for a portable, affordable, and easy-to-use device for cervical pre-cancer detection, especially in low-resource settings. We have developed a dual-modality fiber-optic endoscope system (SmartME) that integrates high-resolution fluorescence imaging (FLI) and quantitative diffuse reflectance spectroscopy (DRS) onto a smartphone platform. The SmartME consists of a smartphone, a miniature fiber-optic endoscope, a phone attachment containing imaging optics, and a smartphone application (app). FLI is obtained by painting the tissue with a contrast agent (e.g., proflavine), illuminating the tissue and collecting its fluorescence images through an imaging bundle that is coupled to the phone camera. DRS is achieved by using a white LED, attaching additional source and detection fibers to the imaging bundle, and converting the phone camera into a spectrometer. The app collects images/spectra and transmits them to a remote server for analysis to extract the tissue parameters, including nuclear-to-cytoplasm ratio (calculated from FLI), concentrations of oxyhemoglobin (HbO2) and deoxyhemoglobin (Hb) as well as scattering (measured by DRS). These parameters can be used to detect cervical dysplasia. Our preliminary studies have demonstrated that the SmartME can clearly visualize the nuclei in living cells and in vivo biological samples, with a high spatial resolution of ~3.1?m. The device can also measure tissue absorption and scattering properties with comparable accuracy to those of a benchtop DRS system. The SmartME has great potential to provide a compact, affordable, and 'smart' solution for early detection of neoplastic changes in cervix.

10057-11, Session 3

Characterizing breast lesions using multimodal diffused optical tomography, magnetic resonance imaging and elastography

Bin Deng, Bo Zhu, Amir Y. Sajjadi, Stefan A. Carp, Athinoula A. Martinos Ctr. for Biomedical Imaging (United States)

Developing biomarkers for the early and accurate prediction of breast cancer neoadjuvant chemotherapy (NACT) outcomes is needed to tailor treatment on an individual basis for improved disease free and overall survival. However, due to the fact that breast cancers are complex, evolving systems characterized by profound spatial and temporal heterogeneity in their biological nature and responses to treatment, individual functional biomarkers that depict only one aspect of tumor physiology are intrinsically limited. In this work, we present a multimodal breast imaging system that is comprised of an 8-channel magnetic resonance (MR) compatible diffuse optical tomographic (DOT) unilateral breast coil with integrated MR elastography (MRE) actuator. A TechEn CW6 Imager that offers 32 continuous wave (16 at 690 nm and 16 at 830 nm) optical sources and 32 avalanche photodiode detectors is used to acquire optical images concurrently with routine breast MRI scans that include high-resolution 3D T1-weighted and dynamic contrast-enhanced MRI (DCE-MRI) sequences using a Siemens 3T scanner. An electromechanical MRE actuator is powered by an audio power amplifier to play back 100 Hz sinusoidal shear waves that propagate through breast tissues. We demonstrate the MRI structure-guided optical image reconstruction as well as the derivation of functional biomarkers from DCE-MRI and elastogram on breast cancer patients. Our results show that a rich set of structural and functional biomarkers quantified from complementary imaging technologies can be used to characterize breast lesions. The multimodal DOT/MRI/MRE imaging platform could be an effective tool for the comprehensive assessment of underlying pathophysiological responses of breast tumors to NACT.

10057-12, Session 3

Real-time fluorescence T/B ratio calculation in multimodal endoscopy for detecting GI tract cancer

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Multimodal endoscopy, with fluorescence-labeled peptides binding to overexpressed molecular targets, is a promising technology to visualize early-stage cancer. Target/background ratio (T/B) is the quantitative analysis used to correlate fluorescence regions to cancer. Currently,

T/B calculation is slow and does not provide real-time feedback to the endoscopist. To achieve real-time computer assisted diagnosis (CAD), we establish image processing protocols for calculating T/B and locating high-risk fluorescence regions for guiding biopsy and therapy in Barrett's esophagus patients.

Methods: Chan-Vese algorithm, an active contour model, is used to segment high-risk regions in fluorescence videos. The energy function of this algorithm contains the penalty on length of the contour, area inside the contour, and variance of intensity inside and outside the contour. A semi-implicit gradient descent method was applied to minimize the energy function and evolve the segmentation. The surrounding background was then identified using erosion and dilation. The average T/B ratio was computed and displayed based on user-selected thresholding. Evaluation was conducted on 25 fluorescence videos acquired from clinical video recordings using a custom multimodal endoscope.

Results: With a processing speed of 2 fps on a laptop computer, we obtained accurate segmentation of high-risk regions examined by experts. For each case, the clinical user could optimize target boundary by changing the penalty on area inside the contour.

Conclusion: CAD system of identifying high-risk regions of early esophageal cancer was developed. Future work will increase processing speed to >5 fps, refine the clinical interface, and apply to additional GI cancers and fluorescence peptides.

10057-13, Session 3

Real-time 3D Optical shape sensing for in-body navigation *(Invited Paper)*

Merel Leistikow, Philips Research (Netherlands)

No Abstract Available

10057-14, Session 3

High spatial denisty, multi-modal diffuse optical tomography of breast cancer

Jeffrey M. Cochran, Univ. of Pennsylvania (United States); David R. Busch, The Children's Hospital of Philadelphia (United States) and Univ. of Pennsylvania (United States); Han Y. Ban, Venkaiah C. Kavuri, Univ. of Pennsylvania (United States); Martin J. Schweiger, Simon R. Arridge, Univ. College London (United Kingdom); Arjun G. Yodh, Univ. of Pennsylvania (United States)

We present high spatial density, multi-modal, parallel-plate Diffuse Optical Tomography (DOT) imaging systems for the purpose of breast tumor detection. One hybrid instrument provides time domain (TD) and continuous wave (CW) DOT at 64 source fiber positions. The TD diffuse optical spectroscopy with PMT- detection produces low-resolution images of absolute tissue scattering and absorption while the spatially dense array of CCD-coupled detector fibers (108 detectors) provides higher-resolution CW images of relative tissue optical properties. Reconstruction of the tissue optical properties, along with total hemoglobin concentration and tissue oxygen saturation, is performed using the TOAST software suite. Comparison of the spatially-dense DOT images and MR images allows for a robust validation of DOT against an accepted clinical modality. Additionally, the structural information from co-registered MR images is used as a spatial prior to improve the quality of the functional optical images and provide more accurate quantification of the optical and hemodynamic properties of tumors. We also present an optical-only imaging system that provides frequency domain (FD) DOT at 209 source positions with full CCD detection and incorporates optical fringe projection profilometry to determine the breast boundary. This profilometry serves as a spatial constraint, improving the quality of the DOT reconstructions while retaining the benefits of an optical-only device. We present initial images from both human subjects and phantoms to display the utility of high spatial density data and multi-modal information in DOT reconstruction with the two systems.

10057-15, Session 3

Novel hybrid technology for early diagnostics of sepsis

Inga Saknite, Andris Grabovskis, Uldis Rubins, Zbignevs Marcinkevics, Edgars Kviesis-Kipge, Sigita Kazune, Janis Spigulis, Univ. of Latvia (Latvia)

The aim of this study was to develop a hybrid technology for early diagnostics and treatment monitoring of sepsis by means of hyperspectral and thermal imaging. Sepsis is harmful body's response to infection which causes inflammatory reactions throughout the body that can lead to tissue damage, organ failure and death. Severe sepsis causes poor organ function or insufficient blood flow and it accounts for a third of patient deaths in intensive care unit. Mortality rates for patients with septic shock can be up to 80%. This novel technology is designed in collaboration with medical doctors and is meant to be used in intensive care units for early diagnostics of sepsis thus making it possible to treat the disease as early as possible.

The developed hybrid technology consists of a snapshot hyperspectral imaging camera (Ximea, sensitivity in the spectral range of 470-630 nm) and a thermal camera. This joint approach focuses on visualization and quantification of tissue microhemodynamics – skin mottling and oxygen utilization disorders are quantified by analyzing hyperspectral images of tissue while microperfusion heterogeneity is assessed by thermal images. This novel technology is noninvasive, can obtain data at patient's bedside and can reveal changes in oxygen saturation and microperfusion in real time.

This technology was tested in laboratory environment on 15 healthy volunteers and also in clinics on sepsis patients in intensive care unit. Preliminary results show that this hybrid technology provides useful information for medical doctors for early diagnostics and monitoring of sepsis progression non-invasively in real time at patient's bedside.

10057-16, Session 4

New contrasts for x-ray imaging and synergy with optical imaging *(Invited Paper)*

Ge Wang, Rensselaer Polytechnic Institute (United States)

Due to its penetrating power, fine resolution, unique contrast, high-speed, and cost-effectiveness, x-ray imaging is one of the earliest and most popular imaging modalities in biomedical applications. Current x-ray radiographs and CT images are black and white, since they reflect overall energy attenuation. Recent advances in x-ray detection and image reconstruction technologies have changed our perception and expectation of x-ray imaging capabilities, and generated an increasing interest in imaging biological soft tissues in terms of energy-sensitive material decomposition, x-ray induced fluorescence and luminescence, phase-contrast, and small angle scattering properties. Such novel x-ray imaging modes are potentially mendable for hybridization with optical molecular tomography. In this presentation, some recent work at our Biomedical Imaging Center will be reported, and several ideas will be shared including x-ray modulated opto-genetics (x-optogenetics).

10057-17, Session 4

Combined multispectral spatial frequency domain imaging and computed tomography system for intraoperative breast tumor margin assessment

David M. McClatchy III, Venkataramanan Krishnaswamy, Stephen C. Kanick, Jonathan T. Elliott, Thayer School of Engineering at Dartmouth (United States); Wendy A. Wells M.D., Richard J. Barth M.D., Dartmouth Hitchcock Medical Ctr. (United States); Keith D. Paulsen, Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

There is a dire clinical need for surgical margin guidance in breast conserving therapy (BCT). We present a multispectral spatial frequency domain imaging (SFDI) system, spanning the visible and near-infrared (NIR) wavelengths, combined with a shielded X-ray computed tomography (CT) system, designed for intraoperative breast tumor margin assessment. While the CT can provide a volumetric visualization of the tumor core and its spiculations, the co-registered SFDI can provide superficial and quantitative information about localized changes tissue morphology from light scattering parameters. These light scattering parameters include both model-based parameters of sub-diffusive light scattering related to the particle size scale distribution and also textural information of the high spatial frequency reflectance. Because the SFDI and CT components are rigidly fixed, a simple transformation can be used to simultaneously display the SFDI and CT data in the same coordinate system. This is achieved through the Visualization Toolkit (vtk) file format in the open-source Slicer medical imaging software package. A complete system analysis, co-registered SFDI-CT data of anthropomorphic phantoms, and preliminary human specimen data will be presented. The ultimate goal of this work is to evaluate this technology in a prospective clinical trial, and the current limitations and engineering solutions to meet this goal will also be discussed.

10057-18, Session 4

Multimodal non-contact photoacoustic imaging and optical coherence tomography using all optical detection

Thomas Berer, Elisabeth Leiss-Holzinger, Research Ctr. for Non Destructive Testing GmbH (Austria)

We present a multimodal optical setup, allowing non-contact photoacoustic imaging (PAI) and optical coherence tomography (OCT). OCT is a fast and non-contact imaging method that allows acquisition of depth-resolved images of subsurface features in turbid media. The method is sensitive to changes in the specimen's refractive index, thereby offering complementary information to photoacoustic signals, which are induced by light absorption. A multimodal setup for OCT and PAI should ideally not rely on any physical contact to a specimen. Thus, commonly used transducers for photoacoustic signal detection, which require acoustic coupling to the specimen, should be avoided. In this work photoacoustic signals are acquired by measuring the surface displacement of a specimen using a fiber-optic Mach-Zehnder interferometer. Photoacoustic signals are excited with a Nd:YAG laser. The interferometer for non-contact PAI detection and the OCT system are realized in the same fiber-optic network. Light from the PAI detection laser and the OCT source are multiplexed into one fiber and the same objective is used for both imaging modalities. Light reflected from specimens is demultiplexed and guided to the respective imaging systems. To allow fast non-contact PAI and OCT imaging the detection spot is scanned across the specimens' surface using a galvanometer scanner. As the same fiber-network and optical components are used for photoacoustic and OCT imaging the obtained images are co-registered intrinsically. Imaging is demonstrated on tissue mimicking and biological samples.

10057-19, Session 4

Multi-segment detector array for hybrid reflection-mode ultrasound and optoacoustic tomography

Elena Mer?ep, iThera Medical GmbH (Germany) and Technische Univ. München (Germany); Neal C. Burton, iThera Medical GmbH (Germany); Xosé Luís Deán-Ben, Daniel Razansky, Helmholtz Zentrum München GmbH (Germany)

The complementary contrast of the optoacoustic (OA) and pulse-echo ultrasound (US) modalities makes the combined usage of these imaging technologies highly advantageous. Due to the different physical contrast mechanisms development of a detector array optimally suited for both modalities is one of the challenges to efficient implementation of a single OA-US imaging device. We demonstrate imaging performance of the first hybrid detector array whose novel design, incorporating array segments of linear and concave geometry, optimally supports image acquisition in both reflection-mode ultrasonography and optoacoustic tomography modes.

Hybrid detector array has a total number of 256 elements and three segments of different geometry and variable pitch size: a central 128-element linear segment with pitch of 0.25mm, ideally suited for pulse-echo US imaging, and two external 64-elements segments with concave geometry and 0.6mm pitch optimized for OA image acquisition. Interleaved OA and US image acquisition with up to 25 fps is facilitated through a custom-made multiplexer unit. Spatial resolution of the transducer was characterized in numerical simulations and validated in phantom experiments and comprises 230 and 300 ?m in the respective OA and US imaging modes.

Imaging performance of the multi-segment detector array was experimentally shown in a series of imaging sessions with healthy volunteers. Employing mixed array geometries allows at the same time achieving excellent OA contrast with a large field of view, and US contrast for complementary structural features with reduced side-lobes and improved resolution.

The newly designed hybrid detector array that comprises segments of linear and concave geometries optimally fulfills requirements for efficient US and OA imaging and may expand the applicability of the developed hybrid OPUS imaging technology and accelerate its clinical translation.

10057-21, Session PSun

Profiling measuring the signal of pulse beat on wrist skin surface by advanced vibrometer interferometer device

Hao-Xiang Lee, National Taiwan Univ. (Taiwan); Shu-Sheng Lee, National Taiwan Ocean Univ. (Taiwan); Yu-Hsiang Hsu, Chih-Kung Lee, National Taiwan Univ. (Taiwan)

With global trends in population aging, the need to decrease and prevent the onset of cardiovascular disease has drawn a lot of attention. However, the cuff-based upper arm traditional sphygmomanometer is still the standard method to retrieve blood pressure information for diagnostic use. It is not easy to be adapted by patients and is not comfortable enough to perform a long term monitoring process. In order to correlate the arterial pulse beating profile on the wrist with blood pressure, an Advanced Vibrometer Interferometer Device (AVID) system is adopted to measure the vibration amplitude of skin and compare with blood pressure measured from the upper arm. The AVID system can measure vibration and remove the directional ambiguity by using circular polarization interferometer technique with two orthogonal polarized light beams. The displacement resolution of the system is nearly 1.0 nm. Since this method is based on optical method, it is cuffless, non-invasive and can perform continuous measurement. By using this method, the correlations between the skin

vibration amplitude and the actually blood pressure is studied. The success of this method could potentially be applied to develop a portable wrist blood pressure monitor system for diagnostics.

10057-22, Session PSun

A single-pixel camera video ophthalmoscope

Benjamin Lochocki, Adrian Gambin, Silvestre Manzanera, Lab. de Óptica Univ. de Murcia (Spain); Esther Irles, Enrique Tajahuerce, Jesús Lancis, Univ. Jaume I (Spain); Pablo Artal, Lab. de Óptica Univ. de Murcia (Spain)

There are several ophthalmic devices, which are able to image the retina, from fundus cameras capable to image the fundus to its entire extent through to scanning ophthalmoscopes with photoreceptor resolution. Unfortunately, latter devices are prone to a variety of ocular conditions like defocus and opacities, which usually degrade the quality of an image. Here, we demonstrate a novel approach to image the retina in real-time using a single pixel camera, which might have the ability to circumvent those optical restrictions. The imaging procedure in the experimental double pass system is a follows: A set of so-called Hadamard patterns is projected rapidly onto the retina using a digital micro mirror device (DMD, f = 22.7 kHz) and covers around 15 visual degrees of the retina. At the same time, the inner product's intensity is measured for each pattern with a photomultiplier tube. Subsequently, an image can be reconstructed computationally. Obtained image resolution is up to 128 x 128px with a varying real-time video frame rate up to 11fps. Experimental results obtained in an artificial eye confirm the tolerance against defocus compared to a conventional multi-pixel array based system. Furthermore, the use of a multiplexed illumination offers a SNR improvement leading to a lower illumination of the eye and hence an increase in patient's comfort. In addition, the proposed system could enable imaging in wavelength ranges where cameras are not available.

10057-23, Session PSun

EEG microstates are associated with functional NIRS resting brain networks

Olajide M. Babawale, The Univ. of Texas at Arlington (United States); Thien Nguyen, Gwangju Institute of Science and Technology (Korea, Republic of); Amarnath S. Yennu, The Univ. of Texas at Arlington (United States); Jae Gwan Kim, Gwangju Institute of Science and Technology (Korea, Republic of); Hanli Liu, The Univ. of Texas at Arlington (United States)

The electrophysiological correlates of hemodynamic resting-state networks (RSNs) are yet to be completely understood in the literature. EEG Microstates, which are maps of EEG scalp topographies that remain stable for a brief period of time (80-120ms), have been proposed as neural signatures of BOLD FMRI-RSNs. However, the relationship between EEG microstates and FNIRS brain networks has not been investigated. In this study, we collected simultaneous EEG-FNIRS whole brain data from 16 young subjects (mean age = 23 ? 2.7 years) in resting (eyes-open) state. After preprocessing of the multimodal data, we used a clustering algorithm to identify EEG microstates across all subjects. In addition, we performed a group ICA on the hemodynamic data to identify FNIRS-RSNs that were active across subjects. The time series for all microstates were each convolved with a double-gamma hemodynamic response function (HRF), and then compared to independent component (IC) time series of FNIRS-RSNs using statistical analyses. We identified 6 EEG microstates and 4 independent FNIRS –RSNs from the simultaneously acquired data. Each EEG microstate was associated with several FNIRS-RSNs. Specifically, each of the microstates were associated with one or more of sensorimotor, attention, default-mode, and executive control networks. Our results are consistent with simultaneous EEG-FMRI studies of resting state networks. These results show that EEG microstates are representative of hemodynamic brain networks. Furthermore, we show that the brain networks that are active at rest are represented in both EEG and FNIRS data. Finally, simultaneous EEG-FNIRS provides complementary information that is beneficial to the study of brain function in the resting state.

10057-24, Session PSun

Relationship between EEG frequency bands and functional NIRS resting state brain networks

Olajide M. Babawale, The Univ. of Texas at Arlington (United States); Thien Nguyen, Gwangju Institute of Science and Technology (Korea, Republic of); Amarnath S. Yennu, The Univ. of Texas at Arlington (United States); Jae Gwan Kim, Gwangju Institute of Science and Technology (Korea, Republic of); Hanli Liu, The Univ. of Texas at Arlington (United States)

Multimodal neuroimaging approaches are very useful for a better understanding of human brain function. Simultaneous EEG-fMRI measurements have shown that several EEG frequency bands are associated with BOLD resting state networks. Several oscillations recorded by EEG reflect different cognitive processes taking place in the brain. However, the relationship between EEG frequency bands and functional near infrared spectroscopy (fNIRS)-based resting state networks has not been investigated up until now. In this study, we employed a data-driven approach to identify fNIRS-derived resting state networks and observed their relationships with EEG frequency bands. We collected simultaneous EEG-fNIRS whole brain data from 16 young subjects (mean age = 23 ? 2.7 years) in resting (eyes-open) state. Then, we applied group-level ICA to the hemodynamic data and identified fNIRS-based resting state networks across all subjects. We compared the independent component (IC) time courses of these networks to EEG power time-series of five (5) frequency bands (delta, theta, alpha, beta, and gamma) taken from the subjects. We identified four (4) FNIRS resting-state networks across all subjects: sensorimotor, attention, executive control, and default-mode networks. In addition, each fNIRS network was related to at least one of the EEG frequency bands analyzed in the study. To our knowledge, this is the first study to investigate the relationship between EEG frequency bands and fNIRS networks. In conclusion, we show in this study that EEG oscillations play a major role in maintaining the functional architecture of the brain. In addition, the neuronal activity measured by EEG and hemodynamic information supplied by fNIRS contain similar information, even though they are recorded differently.

10057-25, Session PSun

Application of kernel method in fluorescence molecular tomography

Yue Zhao, Reheman Baikejiang, Changqing Li, Univ. of California, Merced (United States)

Reconstruction of fluorescence molecular tomography (FMT) is an ill-posed inverse problem. Anatomical guidance in the FMT reconstruction can improve FMT reconstruction efficiently. We have developed a kernel method for FMT to incorporate the anatomical guidance robustly and easily. Kernel method is from machine learning for pattern analysis and is an efficient way to incorporate anatomical information. For finite element method based FMT reconstruction, we calculate a kernel function for each finite element node from an anatomical image, such as a micro-CT image. Then the fluorophore concentration at each node is represented by a kernel coefficient vector and the corresponding kernel function. In the FMT forward model, we have a new system matrix by multiplying the sensitivity matrix with the kernel matrix. Thus, the kernel coefficient vector is the unknown

to be reconstructed following a standard iterative reconstruction process. We convert the FMT reconstruction problem into the kernel coefficients reconstruction problem. The desired fluorophore concentration at each node can be calculated accordingly. Numerical simulation studies have demonstrated that the proposed kernel-based algorithm can improve the spatial resolution of the reconstructed FMT images. Phantom experimental results have also shown that the proposed method can result in better reconstruction quality compared with the regularization-based methods. In the proposed kernel method, the anatomical guidance can be obtained directly from the anatomical image and is included in the forward modeling. One of the advantages is that we do not need to segment the anatomical image for the targets and background.

10057-26, Session PSun

Optically integrated photoacoustic and optical coherence tomography based on a low-coherence Michelson interferometer

Zhongjiang Chen, Sihua Yang, South China Normal Univ. (China)

An all-optically noncontact dual-mode imaging system using a single low-coherence Michelson interferometer which simultaneously achieved photoacoustic microscopy (PAM) and optical coherence tomography (OCT) is presented. The pulse laser-induced photoacoustic signals and the back-scattered photons were alternately detected by the Michelson interferometer. The spatial resolution and imaging capability of the dual-mode imaging system were testified by scattering phantoms. Furthermore, in vivo images of the mouse ear demonstrated that the PAM-OCT can provide complementary anatomical and functional information for imaging of biological tissues, which could be the best co-imaging strategy of PAM and OCT in biomedicine

10057-27, Session PSun

Acoustically integrated dual-mode imaging system: combined microwave induced theremoacoustic imaging and ultrasonic imaging

Zhong Ji, Wanling Luo, South China Normal Univ. (China)

In this paper, we present an acoustically integrated dual-mode imaging system that combined microwave induced theremoacoustic imaging and ultrasonic imaging. When biological tissue radiated by pulsed microwave, an ultrasonic wave is generated, that is so-called microwave induced theremoacoustic effect. At the same time, an ultrasonic generated at the piezoelectric transducer that can be served as source of ultrasonic imaging by electromagnetic and elastic resonance effect reported by our previous article. Then the two acoustic waves will be acquired by the same piezoelectric transducer. Due to the different transmission distance, the two acoustic waves will appear in a different position in the time domain, and therefore, the acoustically integrated dual-mode imaging system can be realized. The characteristics of low cost, simultaneous imaging and noninvasion operation make the system a preferred detector for microwave and ultrasonic applications.

10057-28, Session PSun

Multimodal image registration for effective thermographic fever screening

Yedukondala Narendra Dwith Chenna, Univ. of Maryland, College Park (United States); Pejhman Ghassemi, T. Joshua Pfefer, Jon Casamento, Quanzeng Wang, U.S. Food and Drug Administration (United States)

Mitigation of the threat of infectious pandemics such as Ebola virus disease may be possible through mass screening in public places such as airports. Fever screening based on non-contact infrared thermometers and thermographs (IRTGs) represents the only currently viable mass screening approach. An international standard (IEC 80601-2-59) indicates that regions medially adjacent to the inner canthi provide accurate estimates of core body temperature and are preferred sites for fever screening. Therefore, rapid, automated identification of the canthi within facial thermal images may greatly facilitate fever screening. A common method for detecting canthi in a thermal image is through multi-modal image registration (MMIR) of simultaneously captured thermal and white-light images. Thermal images provide temperature information while white-light images are rich with exploitable features for automatic canthi detection.

We conducted a comprehensive study on different registration models using gradient descent optimization with Normalized Mutual Information (NMI) metrics and evaluated their ability to detect canthi. Experimental IRTG and white-light images suggest that small variation in viewpoints, resolution and scaling significantly impact registration accuracy. To improve registration quality, we introduce a coarse-fine registration strategy based on region of interest (ROI) analysis including face, eyes and edge detection. To generate a qualitative performance metric, we selected a set of control points manually from input images and computed mean square error between these points after registration. We observed significant improvement in accuracy of canthi detection using the proposed registration method. Results suggest that robust MMIR may improve IRTG performance for fever screening.

Conference 10058: Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XVII

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10058-1, Session REM

A tribute to Dr. Ron W. Waynant *(Invited Paper)*

Israel Gannot, Tel Aviv Univ. (Israel) and Johns Hopkins Univ. (United States); Ilko K. Ilev, U.S. Food and Drug Administration (United States); Juanita J. Anders, Uniformed Services Univ. of the Health Sciences (United States); Jin U. Kang, Johns Hopkins Univ. (United States)

Ron, our beloved mentor, friend and colleague has passed away on May 7th, 2016. This presentation will follow his life and remarkable achievements. It will describe his work and original developments in three major fields of his interest: his early inventive work on vacuum ultraviolet laser radiation; specialty fiber-optics for laser transmission, especially high-power short-pulse broadband laser delivery of free-electron laser; and up to his latest work on Photobiomodulation. The authors will share their personal experience working with Ron - a Nobel and creative person, however, very humble.

10058-2, Session 1

Fiber-optic tracheal detection device

Brian E. Souhan, Richard Shmel, U.S. Military Academy (United States); Corinne D. Nawn, U.S. Army Institute of Surgical Research (United States)

Poorly performed airway management procedures can lead to a wide variety of adverse events, such as laryngeal trauma, stenosis, cardiac arrest, hypoxemia, or death as in the case of failed airway management or intubation of the esophagus. Current methods for confirming tracheal placement, such as auscultation, direct visualization or capnography, may be subjective or compromised due to clinical presentation, or require additional specialized equipment that is not always readily available during the procedure. Consequently, there exists a need for a non-visual detection mechanism for confirming successful airway placement that can give the provider rapid feedback during the procedure. Based upon our previously presented work characterizing the reflectance spectra of tracheal and esophageal tissue, we developed a fiber-optic prototype to detect the unique spectral characteristics of tracheal tissue by using glass filters to isolate and compare the relative reflectance intensities at specific wavelengths. Device performance was tested by its ability to differentiate ex vivo samples of tracheal and esophageal tissue both with the larynx, trachea and esophagus intact as well as excised and mounted on cork. The device positively detected tracheal tissue 18 out of 19 trials and only 1 false positive out of 19 esophageal trials. Our proof of concept device shows great promise as a potential mechanism for rapid user feedback during airway management procedures to confirm tracheal placement. Ongoing studies are investigating device optimizations of the probe for more refined sensing and in vivo testing.

10058-3, Session 1

Flexible polymer slab waveguides for light-activated therapy

Moonseok Kim, Massachusetts General Hospital (United States); Sheldon J. J. Kwok, Massachusetts General Hospital (United States) and Harvard Medical School (United States) and Massachusetts Institute of Technology (United States); Harvey H. Lin, Massachusetts General Hospital (United States); Dong Hee Lee, KAIST (Korea, Republic of) and Massachusetts General Hospital (United States); Seok Hyun Yun, Massachusetts General Hospital (United States) and Harvard Medical School (United States) and Massachusetts Institute of Technology (United States)

Conventional light-activated therapies, such as photodynamic therapy (PDT), photochemical tissue bonding (PTB), collagen crosslinking (CXL), low-level light therapy (LLLT), and antimicrobial therapy utilize external light sources and light propagation through free space, limiting treatment to accessible and superficial areas of the body. Recent progress has been made in developing biocompatible polymer waveguides to enhance light delivery to deep tissues. To further expand clinical utility, waveguides should be flexible and tough enough to enable use in anatomically difficult-to-reach regions, while having the requisite optical properties to achieve uniform and efficient illumination of the target area. Here, we present a new class of flexible polymer waveguides optimized for uniform light extraction into tissues. Our slab waveguides comprise two designs: first, a flexible polydimethylsiloxane (PDMS) based elastomer for CXL, and second, a tough polyacrylamide and alginate hydrogel for large-area phototherapies. Our waveguides are optically transparent in the visible wavelengths (400-750 nm) and a multimode fiber is used to couple light into the waveguide. We characterized the light propagation through the waveguides and light extraction into tissue, and validated our results with optical simulation. By changing the thickness and scattering properties, uniform light extraction through the length of the waveguide could be achieved. We demonstrate proof-of-concept scleral photo-crosslinking of an ex vivo porcine eyeball for prevention of myopia.

10058-4, Session 1

Ion-selective optical sensor for continuous on-line monitoring of dialysate sodium during dialysis

Manoj K. Sharma, Arjan J. H. Frijns, Technische Univ. Eindhoven (Netherlands); Rajesh Mandamparambil, TNO (Netherlands); Jeroen P. Kooman, Maastricht Univ. Medical Ctr. (Netherlands); David M. J. Smeulders, Technische Univ. Eindhoven (Netherlands)

Patients with end stage renal disease are dependent on dialysis. In most outpatient centers, the dialysate is prepared with a fixed electrolyte concentration without taking in account the inter-individual differences of essential electrolytes (sodium, potassium and calcium). This ‘one-size fits all’ approach can lead to acute and chronic cardiovascular complications in dialysis patients. On-line monitoring of these essential electrolytes will be an important physiological step towards patient specific dialysate leading to individualized treatment. Currently, changes in electrolyte concentrations are indirectly measured by conductivity measurements, which are not ion-specific.

In this paper, we present a novel optical sensor for on-line monitoring of sodium concentrations in dialysate. This sensor is ion-specific and can detect up to a single ion. The working principle is based on the selective fluorescence quenching of photoinduced electron transfer (PET) molecules. The PET molecules when complexed with sodium ions start fluorescing upon laser excitation. The emission intensity is directly correlated to the sodium concentration. To prove the working principle, a micro-optofluidic device has been fabricated in polydimethylsiloxane (PDMS) with integrated optical fibers for fluorescence light collection. The PET molecules are

covalently grafted in the PDMS microchannel for continuous monitoring of the sodium dialysate concentrations. The experimental setup consists of a laser module (?=450 nm) operating at 4.5 mW, a syringe pump to precisely control the sample flow and a spectrometer for fluorescence collection. The performance of the sensor has been evaluated for sodium ions ranging from 0-50 mM. A clear signal and good response time was observed.

10058-5, Session 1

New fiber-based approaches for optical biopsy

Jessie R. Weber, Christophe Rivière, Antoine Proulx, Pascal Gallant, Ozzy Mermut, INO (Canada)

Optical biopsy of tissue using fiber optic probes has proven to be a powerful tool for non-invasive and minimally invasive diagnostics. However, there are still many challenges to improving diagnostic value and commercial translation of these techniques. Many fiber-based methods are limited by background noise, which impairs sensitivity and specificity. Aspects of quality control, such as adequacy of the target of interest sampled and validation of optical measurements with histopathology can be problematic. Complexity, cost, and disposability or sterilizability are roadblocks to widespread clinical use. Here, we present new approaches to using fibers for optical biopsy aimed at solving these problems. Specifically, the new concepts are designed with the goals of being simple and disposable, to improve control of light delivery and collection from the sample, and to inherently enable better quality control of the biopsy process. A concept-of-operation aimed at nearly zero impact to the work flow of the biopsy and standard pathology procedures will be outlined. Several concepts for fiber implementations will be presented. A trade-off analysis of the concepts used to select a first implementation for testing will be presented. Preliminary experimental validation in phantoms and tissue samples will be presented for the selected configuration.

10058-6, Session 1

Ultraviolet spectroscopic breath analysis using hollow-optical fiber as gas cell

Takuro Iwata, Takashi Katagiri, Yuji Matsuura, Tohoku Univ. (Japan)

A breath analysis system based on ultraviolet absorption spectroscopy was developed by using a hollow-optical fiber as gas cell.The hollow optical fiber functions as a long path and extremely small volume gas cell.Firstly we evaluated the measurement sensitivity of the system by using NO gas as a gas sample.NO gas with 50 ppb concentration was successfully measured with the system with a laser-driven, high intensity light source and a 3-meter long, aluminum-coated hollow optical fiber.Then we measured and analyzed an absorption spectrum of breath sample in ultraviolet region with wavelengths region of around 200-300 nm.We found that the main absorbing components were H2O, isoprene, and O3 converted from O2 by radiation of ultraviolet light.Then we estimated the concentration of isoprene in breath by using multiple linear regression analysis as around 180 ppb.It was shown that such trace components with a concentration lower than 1 ppm could be quantitatively detected by using the long hollow-optical fiber and high-intensity ultraviolet light source.

10058-7, Session 2

Fiber Lasers for medical diagnostics and treatments: state of the art, challenges and future perspectives *(Keynote Presentation)*

Stefano Taccheo, Swansea Univ. (United Kingdom)

Fiber laser is a fast growing yet quite young type of laser with huge potential in healthcare due to versatility and reliability. The talk discusses present and future applications of fiber lasers to medical diagnostic and treatments. It first reviews the state of the art with particular emphasize on diagnostic, spectroscopy, ophthalmology and surgery. The talk discusses some of the challenges encountered, as the need for active feedback for treatments and the missing of appropriate wavelength for diagnostic. A roadmap for the development of the next generation of fiber lasers is presented and synergies between sensing and new lasers are discussed.

10058-8, Session 3

In-situ monitoring of drug release from therapeutic eluting polyelectrolyte multilayers by enhanced long-period fiber grating

Fan Yang, Stevens Institute of Technology (United States); Jiri Kanka, Institute of Photonics and Electronics of the ASCR, v.v.i. (Czech Republic); Jouha Min, Paula T. Hammond, Massachusetts Institute of Technology (United States); Henry Du, Fei Tian, Stevens Institute of Technology (United States)

Layer-by-layer (LbL) polyelectrolyte thin film is an attractive controlled drug delivery method with its ability to tune the drug incorporation and release profiles. Herein, long-period grating (LPG) based lab-on-fiber platform is utilized for in-situ time-resolved study of the kinetics and mechanism of therapeutic release from antibiotic drug- and growth factor-laden polyelectrolyte coatings deposited by layer-by-layer (LbL) assembly. This lab-on-fiber platform structure is sensitive to LbL events at monolayer level, which allows continuous measurements of release profiles. Therapeutic-eluting polyelectrolyte polymer films deposited on implantable medical devices and components via LbL assembly for controlled release of therapeutic agents as part of the patient-care strategy are at the frontier of the ever-expanding field of LbL research. Their development and ultimate utility as clinical solutions can be greatly facilitated by the ability to measure the release profiles of the therapeutics in situ.

10058-9, Session 3

Comparison of probes for laser tumor ablation with integrated distributed temperature sensing

Riccardo Gassino, Politecnico di Torino (Italy); Emiliano Schena, Univ. Campus Bio-Medico (Italy); Daniele Tosi, Nazarbayev Univ. (Kazakhstan); Alberto Vallan, Guido Perrone, Politecnico di Torino (Italy)

Thermotherapies, and laser ablation (LA) in particular, are gaining an increasing popularity as less invasive alternatives to surgical resection in the cure of solid tumors. According to medical literature, optimal treatments would require not only an irradiation pattern matched with tumor shape to minimize the intervention duration, but also the knowledge of some key indicators of the outcome, the most important being the induced temperature to guarantee the cell necrosis while avoiding carbonizations. Temperature, however, is not easy to predict a priori from power and exposure time due to the strong dependence on the tissue composition and the possible presence of blood vessels. Moreover, given the large gradients involved, temperature should ideally be known in different points within the ablation area, requiring therefore distributed or quasi-distributed measurement systems. In the last BIOS/Photonics West conferences we presented an innovative probe for LA that makes use of a double cladding fiber with micro-structured surface to shape the laser beam irradiation pattern and with integrated fiber Bragg gratings to sense the temperature.

This paper describes the latest evolutions of such a probe and reports its characterization in different operative situations. First, a study with models and experiments is conducted to analyze the relation between induced temperature profile and values read by the probe for various irradiation pattern shapes. Then, different approaches to realize a distributed or quasi-distributed temperature sensing using multiplexed and chirped gratings are experimentally tested in phantoms made with ex-vivo animal livers to mimic real application situations.

10058-10, Session 3

Proton therapy dosimetry by using silica glass optical fiber microprobes

Arash Darafsheh, Univ. of Pennsylvania (United States); Reza Taleei, Univ. of Texas SW Medical Ctr. (United States); Alireza Kassaee, Jarod C. Finlay, Univ. of Pennsylvania (United States)

We showed the feasibility of clinical proton beam dosimetry using bare silica glass fibers. The spectrum of such fiber irradiated with medical proton beams shows two distinct peaks at 450 and 650 nm, whose spectral shape is different from that of ?erenkov radiation. We believe that the nature of these peaks is related to the point defects of silica. The emission at 650 nm is correlated with the radiation absorbed dose form the proton beam in agreement with measurements performed by a standard ion chamber.

10058-11, Session 4

Scintillating fiber optic dosimeters for breast and prostate brachytherapy

Luis Moutinho, Univ. de Aveiro (Portugal)

Brachytherapy is a radiotherapy modality where the radioactive material is placed close to the tumour. It is a common treatment for breast and prostate cancers. These treatments can be low-dose-rate using isotopes with mean energy of 30 keV or high-dose-rate using isotopes such Ir-192 with a mean energy of 380 keV. Currently these treatments are performed without dosimetry for quality control and quality assurance.

We developed a dosimeter based on scintillating optical fibre's allowing real-times dosimetry and real-time dose correction in breast and prostate brachytherapy.

One major concern when using plastic materials for dosimetry is their low threshold for Cherenkov radiation. Cherenkov radiation is a bluish light originated by charged particles traveling through a medium with a velocity higher than the velocity of light in that medium. Iridium -192 decays by emitting beta particles and gamma radiation where a gamma photon with an average energy of 0.38 MeV (max 1.06 MeV) is released in the process and by electron capture (4%). Once the Cherenkov threshold for PMMA is lower than the Ir-192 energy, some solutions were considered.

We characterized two sensitive probes with 1 mm and 0.5 mm diameter, the former allowing insertion in a typical applicator seed implant needle (17 ga) used in brachytherapy.

A prototype was developed using state of the art in silicon photomultipliers and dedicated electronics. Clinical studies were performed in collaboration with two Portuguese hospitals for LDR and HDR regimes.

10058-12, Session 4

SMART micro-scissors with dual motors and OCT sensors

Chaebeom Yeo, Seonjin Jang, Hyun-cheol Park, DGIST (Korea, Republic of); Peter L. Gehlbach, The Johns Hopkins Univ. School of Medicine (United States); Cheol Song, DGIST (Korea, Republic of)

Various end-effectors of microsurgical instruments have been developed and studied. Also, many approaches to stabilize the tool-tip using robotics have been studied such as the steady hand robot system, Micron, and SMART system. In our previous study, the horizontal SMART micro-scissors with a common path swept source OCT distance and one linear piezoelectric (PZT) motor was demonstrated as a microsurgical system. Because the outer needle is connected with a mechanical handle and moved to engage the tool tip manually, the tool tip position is instantaneously changed during the engaging. The undesirable motion can make unexpected tissue damages and low surgical accuracy. In this study, we suggest a prototype horizontal SMART micro-scissors which has dual OCT sensors and two motors to improve the tremor cancellation. Dual OCT sensors provide two distance information. Front OCT sensor detects a distance from the sample surface to the tool tip. Rear OCT sensors gives current PZT motor movement, acting like a motor encoder. The PZT motor can compensate the hand tremor with a feedback loop control. The manual engaging of tool tip in previous SMART system is replaced by electrical engaging using a squiggle motor. Compared with previous study, this study showed better performance in the hand tremor reduction. From the result, the SMART with automatic engaging may become increasingly valuable in microsurgical instruments.

10058-13, Session 4

Toolkit for multiplexed sensing of physiological parameters in the distal lung with fluorescent probes on multicore fibres

Tushar R. Choudhary, The Univ. of Edinburgh (United Kingdom) and Heriot-Watt Univ. (United Kingdom); Michael G. Tanner, Heriot-Watt Univ. (United Kingdom); Alicia Megia-Fernandez, The Univ. of Edinburgh (United Kingdom); Kerrianne Harrington, Harry A. Wood, Univ. of Bath (United Kingdom); Sunay Chankeshwara, Patricia Zhu, The Univ. of Edinburgh (United Kingdom); Debaditya Choudhury, Heriot-Watt Univ. (United Kingdom); Fei Yu, Univ. of Bath (United Kingdom); Robert R. Thomson, Rory R. Duncan, Heriot-Watt Univ. (United Kingdom); Kevin Dhaliwal M.D., Mark Bradley, The Univ. of Edinburgh (United Kingdom)

We present a toolkit for a multiplexed pH and oxygen sensing probe in the distal lung using multicore fibres. Measuring physiological relevant parameters like pH and oxygen is of significant importance in understanding changes associated with disease pathology. We present here, a single multicore fibre based pH and oxygen sensing probe which can be used with a standard bronchoscope to perform in vivo measurements in the distal lung.

The multiplexed probe consists of fluorescent pH sensors (fluorescein based) and oxygen sensors (Palladium porphyrin complex based) covalently bonded to silica microspheres (10 μm) loaded on the distal facet of a 19 core (10 μm core diameter) multicore fibre (total diameter of ~150 μm excluding coating). Pits are formed by selectively etching the cores using hydrofluoric acid, multiplexing is achieved through the self-location of individual probes on differing cores. This architecture can be expanded to include probes for further parameters. Robust measurements are demonstrated of self-referencing fluorophores, not limited by photobleaching, with short (100ms) measurement times at low (~10μW) illumination powers.

We have performed on bench calibration and tests of in vitro tissue models and in an ovine whole lung model to validate our sensors. The pH sensor is demonstrated in the physiologically relevant range of pH 5 to pH 8.5 and with an accuracy of ± 0.05 pH units. The oxygen sensor is demonstrated in gas mixtures downwards from 20% oxygen and in liquid saturated with 20% oxygen mixtures (~8mg/L) down to full depletion (0mg/L) with ~0.5mg/L accuracy.

10058-14, Session 4

Interferometric and localized surface plasmon based fiber optic sensor

Harald Ian D. Muri, Andon Bano, Dag Roar Hjelme, Norwegian Univ. of Science and Technology (Norway)

Already at an early stage of the fiber-optic (FO) sensor era, several FO systems utilized Fiber Bragg Gratings (FBG), interferometric cavities, or attenuation of light for multiplexed sensing. The multiplexed FO systems usually require one single point for one sensing parameter and another single point for another sensing parameter. However, in some applications as in the medical field, there is a great need for many sensing parameters in one single point only. Fiber optic sensors exploiting optical probes such as noble metal nanoparticles (NMNP) have potential to obtain several sensing parameters in one single point. This is possible by spectrally resolving the local surface plasmon resonance (LSPR) frequencies that NMNP of different shape and size exhibit. Various single point FO LSPR based sensors proposed over the last decade applies LSPR interacting with the evanescent field around the fiber core or with the light at fiber end face. To the best of our knowledge, there are no proposals yet of any single point and multiparameter FO sensors that is based on interferometry and LSPR. In this paper we report on a single point, multiparameter, LSPR and interferometric based FO sensor architecture where gold nano-urchins (GNU) are immobilized in an acrylamide based stimuli-responsive hydrogel droplet on the fiber end face. The results from proof-of-principle concept experiments presents LSPR sensing of refractive index occurring in the visible (VIS) range and interferometric measurements of volumetric changes of the stimuli-responsive hydrogel occurring in the infrared (IR) range.

10058-15, Session 4

Highly sensitive and selective biosensor based on graphene oxide coated long period grating

Mohammed Saad Shaikh, Chen Liu, Bangor Univ. (United Kingdom); Matthew C. Partridge, Stephen W. James, Cranfield Univ. (United Kingdom); WeiDong Zhu, Saint Peter's Univ. (United States); Xianfeng Chen, Bangor Univ. (United Kingdom)

We propose an optical fiber immunosensor based on graphene oxide coated dual-peak long period grating (GO-dLPG), in which the antibody immobilized GO layer was used as a biointerface as well as a loading platform for biomarkers essential for immunosensing. The kinetics of the antibody/antigen reaction was monitored using a GO-dLPG. The reaction produced a detectable optical signal in terms of variations in the resonant wavelength of the GO-dLPG. The signal obtained was proportional to the analyte concentration. The GO was deposited on dLPG fiber surface. Subsequently, the morphological and optical properties of GO layer were characterized by Raman spectroscopy, scanning electron microscope, atomic force microscopy, and dLPG spectra. The GO-dLPG was biofunctionalized by immobilizing IgG on its surface via EDC/NHS scheme. The performance of GO-dLPG biosensor was evaluated by monitoring the kinetic interaction between IgG and anti-IgG with different concentrations. The sensitivity of GO-based biosensor depends on not only the bulk refractive index (RI) sensitivity of photonic device but also the bio-binding efficiency at biointerface. The bulk RI sensitivity of dLPG was enhanced 240% by GO deposition. The bio-binding efficiency depended on the properties of sensing surface, types of analyte, and the number of binding cites. The presented GO-dLPG biosensor achieved a detectable analyte concentration of 0.015 mg/L. The selectivity was validated by non-reaction between IgG and analyte (anti-PSA). The GO functionalized fibre grating sensor has the prospects of a promising photonic biosensor with the advantages of miniaturization, label free, real time detection as well as high sensitivity, specificity, reusability and low limit of detection.

10058-16, Session 4

Fibre optic time-resolved spectroscopy using CMOS-SPAD arrays enables lung disease diagnosis

Katjana Ehrlich, Heriot-Watt Univ. (United Kingdom); Andras Kufcsák, Nikola Krstaji?, Robert K. Henderson, Kevin Dhaliwal M.D., The Univ. of Edinburgh (United Kingdom); Robert R. Thomson, Heriot-Watt Univ. (United Kingdom); Michael G. Tanner, The Univ. of Edinburgh (United Kingdom) and Heriot-Watt Univ. (United Kingdom)

Lung diseases are a great unsolved burden on the healthcare system, in many cases due to a lack of accurate in-situ diagnostics. To attempt to fill this gap, the UK-EPSRC Proteus project seeks to use novel multi-core fibres for multiplexed sensing, and advanced detector technologies for single-photon and time-resolved detection, for the development of a minimally invasive method of identifying pulmonary infections and inflammation for patients in critical care in-situ.

Two primary types of spectroscopy can be used for in-vivo endoscopic detection of physiological parameters, fluorescence-based sensing and molecular reporters (e.g. pMBA) which responses are sensitive to the local environment (eg. pH). However, their emission intensity and amplitude are influenced by background signals, intrinsic and quantitative factors. Many of these problems can in principle be addressed using time-resolved detection. For fluorescence-based sensing, time-resolved spectroscopy can enable interrogation of the local environment via the fluorescence lifetime, which is less sensitive to fluorophore concentration, photobleaching and tissue autofluorescence. This time-resolved detection also enables the removal of any unwanted fibre background through time-gating.

With these basic goals in mind, we constructed a time-correlated single photon counting (TCSPC) spectrograph enabled through CMOS-based Single Photon Avalanche Diodes (SPADs). The detector collects the TCSPC histogram simultaneously from 256x2 pixels with >kHz count rate; with integrated time-gating allowing higher count rate and shorter measurement times. Here we present results for point-sensing of biomedical substrates and physiological parameters such as pH and temperature.

10058-17, Session 4

Femtosecond laser fabrication of fiber based optofluidic platform for flow cytometry applications

Murat Serhatlioglu, Bülend Ortac, Caglar Elbuken, Bilkent Univ. (Turkey); Mehmet E. Solmaz, Izmir Katip Celebi Univ. (Turkey)

Miniaturized optofluidic platforms play an important role in bio-analysis, detection and diagnostic applications. The advantages of such miniaturized devices are extremely low sample requirement, low cost development and rapid analysis capabilities. Fused silica is advantageous for optofluidic systems due to properties such as being chemically inert, mechanically stable, and optically transparent to a wide spectrum of light. As a three dimensional manufacturing method, femtosecond laser scanning followed by chemical etching shows great potential to fabricate glass based optofluidic chips. In this study, we demonstrate fabrication of all-fiber based, optofluidic flow cytometer in fused silica glass by femtosecond laser machining. 3D particle focusing was achieved through a straightforward planar chip design with two separately fabricated fused silica glass slides thermally bonded together. Bioparticles in a fluid stream encounter with optical interrogation region specifically designed to allocate 405nm single mode fiber laser source and three multi-mode collection fibers for forward scattering, side scattering and florescent signals detection. We used LabVIEW-FPGA unit for real-time optical signal processing with pulse processing algorithm and interface to achieve over 2000 events/sec. Also in

real-time, we were able to count number of events, achieve size distribution for polystyrene particles using forward and side scattered light, and detect two different colors using fiber optical wavelength division multiplexing filters. Our platform shows promise for optical and fluidic miniaturization of flow cytometry systems.

10058-18, Session 4

Analysis of anhydrous glucose and human serum assisted by Raman spectroscopy

Naara González Viveros, Héctor Hugo Cerecedo-Nuñez, Patricia Padilla-Sosa, Univ. Veracruzana (Mexico); Aaron Flores-Gil, Manuel May Alarcón, Univ. Autónoma del Carmen (Mexico); Miguel A. Basurto Pensado, Univ. Autónoma del Estado de Morelos (Mexico); Maria Gabriela Alcantara López, Omar David Muñoz Muñiz, Univ. Veracruzana (Mexico)

Raman spectroscopy has been considered like a potentially important clinical tool for real-time diagnosis of disease and evaluation of living tissue, whit the proposal to development noninvasive glucose measurements with lower power than other reported studies, in this work we report experimental tests made with an excitation source of semiconductor laser at 785 nm and 35 mW power. Measurements were made to different glucose concentrations with variation from 50 mg/dL to 6000 mg/dL, for this, three series with different ranges of concentration were analyzed. In the same way measurements of freeze-dried and reconstitute human serum are reported, in which healthy volunteers had 12 hours fasting and non-fasting conditions with its corresponding values of glucose taken through a conventional glucometer. For assisted Raman spectroscopy five spectra per test were obtained and subsequently average was calculated, the spectra were studied in a range of 500 to 1700 cm-1. This work explores the variation in the intensity of the peaks of glucose at 1065 cm-1 and 1127 cm-1 as a function of glucose concentration. In the obtained results there observes a behavior with positive slope in both substances, interrelation being observed between the measurements, being promissory for non-invasive measurement.

10058-20, Session 6

Analysis of propagation properties of terahertz hollow-optical fiber by using time-domain spectroscopy and application for THz wave remote spectroscopy

Kosei Ito, Takashi Katagiri, Yuji Matsuura, Tohoku Univ. (Japan)

Behavior of terahertz pulse propagation in hollow optical fibers is investigated by using terahertz time-domain spectroscopy. Transmission loss spectra of hollow optical fibers made of a flexible polycarbonate tube with inner silver layer are measured at the wavelength rage 0.2 to 3 THz. The spectra of fibers with an inner diameter of 3 mm and the length of 42 cm show some interference peaks around 1-2 THz and it is found that, these are because of mode interference between the lowest order TE11 mode and TM11 mode. The mode mixing is also explained from results of time-frequency analysis performed by Short-time Fourier transform and it is confirmed that the traces of TE11 and TM11 modes clearly appear on loss spectra at 0 to 10 ps after the first signal's detection. Dispersion properties of the transmission modes are derived from measured phases of transmitted pulses and it is found that group velocities in hollow optical fibers decrease in low frequency region. The group velocity curve coincides well with theoretical result of TE11 mode and this suggest that TM11 mode have little influence to the propagation constant of hollow optical fibers. Finally we performed a THz wave remote spectroscopy using the hollow optical fiber and acquired a clear transmission spectrum including absorption peak of the theophylline at around 1 THz.

10058-21, Session 6

Microbend fiber optic sensor for perioperative pediatric vital signs monitoring

Zhihao Chen, Quanzhou Normal Univ. (China); Hwan Ing Hee, KK Women's and Children's Hospital (Singapore); Soon Huat Ng, Ju Teng Teo, Xiufeng Yang, Institute for Infocomm Research (Singapore); Dier Wang, Nanyang Technological Univ. (Singapore)

Monitoring of physiological parameters is essential in ill and perioperative patients to detect deterioration in condition and facilitate appropriate medical intervention. Limitations in current technology include the need for direct skin contact, restriction of patient's movement by cables and sensors and poor compliance. Infants, in particular tolerate placement of multiple monitors poorly. An ideal technology is one that gives real time, noninvasive, contactless, cableless, accurate monitoring for rapid response.

We developed a microbend fiber optic sensor for vital signs monitoring that is free from direct contact with skin, cableless, electromagnetic interference free and low cost. Use of our sensor to monitor respiratory rate and heart rate had been recently demonstrated in adults when placed under a 12 inch mattress. To meet the challenge of small body weight in infants, a highly sensitive sensor is required. The feasibility of our device was studied on infants undergoing surgery with IRB approval. Five participants ranging from one month to 12 months were enrolled. The sensor was placed under a barrier sheet on the operating table and data is wirelessly transmitted to a notebook and displayed. All patients received standard intraoperative monitoring. The results showed good agreement in heart rate and respiratory rate between our device and the standard physiological monitoring with a newly derived algorithm. More infants are expected to be enrolled in the trial. Our new microbend fiber optic sensor is a potential solution for monitoring respiratory rate and heart rate in perioperative or ill infants and is Magnetic Resonance Imaging safe.

10058-22, Session 6

Performance improvement of an all-optical Fabry Perot ultrasound detector

Supriya V. Thathachary, Shai Ashkenazi, Univ. of Minnesota, Twin Cities (United States)

A highly sensitive Fiber-Optic Fabry Perot Ultrasound sensor with a self-written waveguide is presented in this work. A simulated device using Gold mirrors showed periodic resonance with Q factor 1900 for 45 μm thick devices. Including a waveguide to limit lateral power losses resulted in improvement of Q-factor to 3200. Simulations also indicated greater improvement in Q-factor upon the introduction of waveguide with larger device thicknesses.

Subsequently, a prototype was fabricated on a single mode optical fiber. Benzocyclobutene was chosen as the cavity medium as it undergoes a refractive index change upon exposure to UV. The refractive index change in BCB upon UV exposure was studied using a phase grating. Upon confirming that 2-hour exposure produced a change of 0.004, a self-aligned waveguide was written into the cavity. A consequent increase in Q-factor from 2500 to 5200 was seen for an 80 μm thick device.

Simulation studies indicate further improvement when incorporating dielectric Bragg mirrors instead of Gold, with Q-factors of 6400 and 10200 with and without the waveguide. Therefore, the proposed design includes Dielectric Bragg mirrors as well as a self-aligned waveguide.

The fabrication techniques being fairly controlled and automated, this device is highly suitable for mass manufacturing, making is possible to produce as an inexpensive, disposable device. A potential application is to integrate it within a commercial guidewire to create a smart guidewire capable of detecting vascular vessel walls in order to guide interventions for

Chronic Total Occlusions, reducing risk of wall perforation, which is currently an unmet clinical need.

10058-23, Session 6

An inherently temperature insensitive fiber Bragg grating force sensor for in-vivo applications

John W. Arkwright, Flinders Univ. (Australia) and Arkwright Technologies Pty Ltd (Australia); Luke A. Parkinson, Anthony W. Papageorgiou, Flinders Univ. (Australia)

We present a fiber Bragg Grating sensor design that provides an inherently athermal response to a transverse applied force. The active element of the sensor is formed from two fibres helically wound around a common axis each containing an FBG element. The helical winding of the fibres is positioned within the transducer so that the FBG elements are coincident and located at the point where the axes of the fibres are in the orthogonal plane to the base of the sensor. An applied force acting on the sensor deflects the fibres sideways so that the upper FBG is compressed and the lower FBG is stretched causing a differential change in the Bragg wavelengths of each element. For small forces, the differential change in wavelength is linearly proportional to the applied force. A change in temperature causes identical change in Bragg wavelength on both FBG elements and therefore does not affect the differential change caused by the applied force.

Using this design we have reduced the temperature dependence of our FBG pressure sensors from 10 pm per degree C to less than 0.1 pm per degree C, with the residual temperature dependence being largely made up of temperature variations in the solid state spectrometer used to acquire data.

These sensors are ideally suited for forming sensing arrays for monitoring in-vivo pressures and forces where fluctuations in temperature are unavoidable, and have been used successfully for monitoring compression bandages and pressure variations in the upper airway of sleep apnoea patients.

10058-43, Session 6

Fractional laser therapeutics: Assessing the conversion of fundamental fiber-optic-generated laser beam profiles into fractional laser microbeams

Arjun Swaminathan, Abbas Bandukwala, Univ. of Pennsylvania (United States); Ilko K. Ilev, U.S. Food and Drug Administration (United States)

No Abstract Available

10058-24, Session 7

Multi-spectral fiber spectroscopy in 0,4-16μm range for biomedical applications *(Invited Paper)*

Viacheslav Artyushenko D.D.S., art photonics GmbH (Germany)

Various biomedical applications of fiber optics in a broad spectral range 0,4-16μm span from endoscopic imaging and Photo Dynamic Diagnostics (PDD) to laser power delivery for minimal invasive laser surgery, tissue coagulation and welding, Photo Dynamic Therapy (PDT), etc. Present review will highlight the latest results in advanced fiber solutions for a precise tissue diagnostics and control of some therapy methods - for so called "theranostic".

Spectral fiber sensing for label free analysis of tissue composition helps to differentiate malignant and normal tissue to secure minimal invasive, but complete tumor removal or treatment. All key methods of Raman, fluorescence, diffuse reflection & MIR-absorption spectroscopy will be compared when used for the same spot of tissue - to select the most specific, sensitive and accurate method or to combine them for the synergy enhanced effect. The most informative spectral features for distinct organs/tumor can be used to design special fiber sensors to be developed for portable and low cost applications with modern IT-features. Examples of multi-spectral tissue diagnostics promising for the future clinical applications will be presented to enable reduced mortality from cancer in the future.

Translation of described methods into clinical practice will be discussed in comparison with the other method of optical diagnostics which should enhance modern medicine by less invasive, more precise and more effective methods of therapy to be fused with in-vivo diagnostics sensors & systems.

10058-25, Session 7

Chalcogenide glass sensors for bio-molecule detection *(Invited Paper)*

Pierre Lucas, Garrett J Coleman, Christopher Cantoni, The Univ. of Arizona (United States); Shibin Jiang, Tao Luo, AdValue Photonics (United States); Bruno Bureau, Catherine Boussard-Pledel, Johann Troles, Univ. of Rennes I (France); Zhiyong Yang, Jiangsu Normal University (China)

Chalcogenide glasses combine a unique set of properties that make them ideal candidate materials for the design of selective bio-molecule sensors. They are the only class of materials that are transparent over the whole mid-infrared range and simultaneously possess excellent rheological properties for molding and drawing advanced optical elements. Many sensor designs can be produced such as tapered fibers, optical-resonators, micro-structured surfaces, ATR elements etc. The wide infrared transparency can be tailored and optimized to collect the vibrational signature of virtually any molecules and in particular to detect the rich fingerprint of bio-molecules in the 4-12 microns region. High quality detection in this region permits the selective identification of hazardous species such as bacteria and viruses. Here we review the advantages and limitations of chalcogenide glasses as well as materials engineering strategies for designing optics with tailored properties.

10058-26, Session 7

Fiber-Bragg-grating-based heater and temperature sensor for hyperthermia treatment

Corneliu I. Rablau, Kettering Univ. (United States)

We report on the development and characterization of a fiber-optic heater and temperature sensor to be employed for simultaneous single-fiber heating and temperature monitoring in heat-based local therapies such as hyperthermia treatment of tumors. The sensor consists of a tilted Fiber Bragg Grating (TFBG) inscribed in the core of a single-mode fiber and coated with an absorbing layer. The coated TFBG acts simultaneously as a radial fiber diffuser for heating as well as a FBG temperature sensor via the Bragg reflection. The device operates in the 1500 to 1600 nm band. A tunable laser (TL) followed by an Erbium-Doped-Fiber-Amplifier (EDFA) provides the power needed for heat generation, with the flexibility of tuning the wavelength to maximize the absorption by different choices of coating we investigated, while avoiding overlaps with the temperature-dependent

Bragg reflection. A second, low power broad band source (1500 to 1600 nm) is used to provide the input signal needed to monitor the Bragg reflection using an Optical Spectrum Analyzer (OSA). The nominal room-temperature-design wavelength of the Bragg reflection is chosen far enough from the EDFA-amplified signal. For temperature calibration, the device is immersed in a controlled temperature bath, and, with the TL+EDFA off, a Bragg-reflection-wavelength vs temperature calibration curve is constructed. For active real-time heating and temperature measurements, the device is immersed in a liquid-filled capillary tube, and with the TL+EDFA on, the recorded Bragg-reflection wavelengths are converted to temperatures and compared with temperature measurements from conventional fine-point thermocouples as well as from a separate, non-heating FBG.

10058-27, Session 7

Real-time artifact removal for blood vessel detection during intraoperative surgery

Amal Chaturvedi, Shetha A. Shukair, Paul Le Rolland, Mayank Vijayvergia, Hariharan Subramanian, Jonathan W. Gunn, BriteSeed, LLC (United States)

The lack of tactile and visual feedback during minimally invasive surgery increases the complexity of already difficult procedures. These problems can lead to inadvertent damage to blood vessels hidden beneath tissue causing serious health risks to patients and a heavy unreimbursable financial burden to hospitals. Existing intraoperative imaging technologies are either too expensive or too cumbersome to be included into existing surgeries. We have developed a low-cost, contrast agent-free, miniaturized smart dissector, with an LED array and a sensor array on its opposite jaws, which is able to localize, track and quantify a blood vessel wrapped around any given tissue in real time with a high resolution. However, the presence of commonly present artifacts, inducing ambient light and hand motion of the surgeon, affect the signal quality. Additionally, the angular geometry of the jaws creates a variable, angle-dependent, non-uniform illumination pattern on the sensors affecting the spatial and temporal resolutions. In this research, we present novel signal processing methods to identify, analyze and remove these artifacts in real time. It is important to note that the angular artifact is removed without a priori knowledge of the jaw angle. The artifact-free system is able to find blood vessels of size 2-8mm with a resolution of 0.5mm. These techniques have undergone successful ex-vivo validation, and will be tested in-vivo.

10058-28, Session 7

Development of multifunctional fluorescence based endoscopic sensors for pH sensing and enzyme detection

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Multicore fibres (MCFs) are the key technology that has enabled the development of micro-endoscopic imaging instruments. In addition to enabling the “imaging” of tissues in hard to reach areas of the body, however, these fibres also enable multiplexed sensing capabilities – a capability which is still underdeveloped. This sensing capability can be achieved using fluorescent polymers, or microbeads, attached to the distal end of the fibre, but the degree and type of multiplexed sensing is dependent on the protocols by which sensing moeties are attached to the fibre. In this work, we demonstrate the potential of using a photo-cleavable nitrobenzyl cage (NVOC), which is attached to the distal end of a functionalized optical fibre. When cleaved using light with an appropriately chosen wavelength, the cleaved location can be used for the spatially selective attachment of fluorescent moieties to the fibre end. Using this protocol, we are able to demonstrate the photocleaving of the NVOC at specific cores on the fibre with a spatial accuracy of micrometers, and the subsequent attachment of carboxylic subsidiaries of fluorescein – enabling the detection of pH sensitivities in the pH 5.0 - 7.0 region. Furthermore, to demonstrate the potential of this technique for fabricating complex sensing molecules, we also demonstrate the proof-of-principle identification of the enzyme chymotrypsin using an enzyme sensitive tri-peptide sequence that was constructed using a combinatorial chemistry approach. In summary, a unique fabrication protocol for sensor attachment enables us to use the devised optical fibres for pH sensing and enzyme activity detection, both of which have promising applications in medical diagnosis.

10058-29, Session 8

UV-fibers as delivery systems for 213 and 266 nm pulsed laser light

Karl-Friedrich Klein, Philipp Raithel, Technische Hochschule Mittelhessen (Germany); Georg Hillrichs, Hochschule Merseburg (Germany)

No Abstract Available.

10058-30, Session 8

Performance characteristics of continuous multicore fiber optic sensor arrays

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Multicore optical fibers have been examined for various sensing applications. For example, it is possible to measure local bend and twist using such fibers, and even to reconstruct the shape of optical fibers in excess of a meter in length. Optical fiber shape reconstruction allows for a highly compact, flexible and physically robust position sensor that could enable numerous novel motion control applications. In order to perform such precise measurements, it is essential that the optical fiber provides sufficient signal over its length to obtain the required bend and twist information. While Rayleigh scattering from the cores is often sufficient, a much larger and more easily interpreted signal is obtained if reflecting gratings, i.e., Bragg gratings, are inscribed along the cores. As such grating sensors come closer to commercialization, is it important to characterize the performance and sensing capability of such multicore fibers in order to best match their properties to various sensing schemes.

In this work we discuss analysis methods that allow for evaluation of continuous multicore fiber grating arrays. We extract local parameters such as grating strength and phase derivative and we correlate these measurements from one core to another to compare performance for the entire array. We also show the response of our fiber to perturbations such as bends, and compare the response to theoretical models. Our analysis provides guidance and design rules for the use of multicore arrays in various applications that require measurements of fiber bend, shape, and position.

10058-31, Session 8

Evaluation and simulation of the radiation profile of fs-laser fabricated optical fiber diffusers

Christoph Vonach, Matthias Domke, FH Vorarlberg (Austria); Tilmann A. Trebst, LifePhotonics (Germany);

Ronald Sroka, LIFE-Zentrum, Hospital of Univ. of Munich (Germany)

Photodynamic therapy and laser-induced thermotherapy are two medical laser applications in oncology and dermatology where a well-defined volume of tissue has to be irradiated, ideally homogeneously over a length of several centimeters inside the human body. So far optical fibers are used to deliver the light into the human tissue. The challenge is to decouple the light at the end of the fiber. Polymer diffusers, attached to the fiber tip, will be damaged especially at high laser powers. The purpose of this investigation is to directly modify the fiber itself for decoupling.

Here the final part of the fiber was processed over a length of 20 mm using femtosecond laser ablation. In this way, the surface can be shaped and roughened in order to scatter the light out of the fiber. Fiber tips with rough dotted and straight lines at one side, as well as with cylindrical and tapered shapes were fabricated. The light emission profile of these fiber diffusors was simulated using a two dimensional MATLAB based model and the commercial ray tracing software LightTools. In order to verify the simulation model, the light of a diode laser was coupled into the fiber and the diffuse area was observed with a gray value camera. The radiation profile was determined by analyzing the brightness of the pixels along the fiber. The results suggest that homogeneous radiation profile with high efficiency of more than 90 % can be achieved This technique may allow for reproducible manufacturing of high power cylindrical diffusors.

10058-32, Session 8

Fabrication of bundle-structured tube-leaky optical fibers for infrared thermal imaging

Takuya Kobayashi, Takashi Katagiri, Yuji Matsuura, Tohoku Univ. (Japan)

Hundreds of tube-leaky optical fibers having a bundled structure were fabricated by glass drawing technique for endoscopic infrared-thermal imaging. The bundle fibers were made of borosilicate glass and have a structure like a photonic crystal fiber having multiple hollow cores. Fabricated fibers have a length of 90 cm and each pixel sizes are less than 80 μm. In these tube-leaky optical fibers, by setting the thickness of glass wall to a quarter-wavelength optical thickness, light is confined in the air core as a leaky mode with a low loss owing to the interference effect of the thin glass wall. We firstly measured the transmission losses of bundled fibers and found that bundled tube-leaky fibers have low transmission losses in spite of the small pixel size. The reason of this seems that the light that leaking out from the core transmits again in the next ring cores. We then tried to deriver thermal images with the bundled fibers combining with an InSb infrared camera having a detection wavelength range of 3-5 μm that coincides with low-loss region of the fibers. Although the observed resolution rapidly degraded near the incident end of the bundle, it became constant at transmitted lengths larger than 10 cm. This is because lossy high order mode excited in the fibers are lost nearby the input end and only low order modes with small leaky losses survive and deliver the image. As a result with the bundle fiber of 30-cm long, we detected the thermal images of a finger with a temperature of about 32 degree C.

10058-33, Session 8

Chalcogenide fibers for mid-infrared molecular sensor probes *(Invited Paper)*

Angela B. Seddon, Harriet A. Parnell, D. Jayasiura, David Furniss, Zhuoqi Tang, Trevor M. Benson, The Univ. of Nottingham (United Kingdom)

Ge-As-Se-Te and Ge-Sb-Se fibres are being fabricated to act as sensor probes for molecular samples, including biological tissue. Characterisation of the glasses and fibres will be presented. These two glass systems present different optical windows and these will be compared and contrasted. Optical loss of the fibres improves with increasing purification. Results of initial mid-infrared sensing will be presented.

10058-19, Session 9

Development of a cylindrical diffusing optical fiber probe for pancreatic cancer therapy

Sangyeob Lee, Yonsei Univ. (Korea, Republic of); Gaye Park, Taihan Fiberoptics Co., Ltd (Korea, Republic of); Jihoon Park, Sungkon Yu, Myungjin Ha, Seulki Jang, Yonsei Univ. (Korea, Republic of); Chihwan Ouh, Chang Hyun Jung, Taihan Fiberoptics Co., Ltd. (Korea, Republic of); Byungjo Jung, Yonsei Univ. (Korea, Republic of)

Although the patient with cancer on pancreas or pancreaticobiliary duct have been increased, it is very difficult to detect and to treat the pancreatic cancer because of its low accessibility and obtuseness. Normally, the pancreatic cancer has been diagnosed using ultrasonography, blood test, CT (computed tomography), ERCP (endoscopic retrograde cholangiopancreato-graphy), EUS (endoscopic ultrasonography) and etc. Phtodynamic therapy (PDT) can be a good method for pancreatic cancer therapy when an appropriate light excite photosensitizer. Light can be delivered to the target by optical fiber through the ERCP or EUS. Optical diffusing fibers have been developed with various methods but many of them have mechanical and biological disadvantage for using on small radius condition such as ERCP or EUS. In this study, we developed therapeutic cylindrical diffusing optical fiber probe (CDOFP) for PDT based on ERCP which has moderate flexibility to treat the cancer on pancreaticobiliary duct or pancreas. The CDOFP consists of biocompatible Teflon tube, multimode glass fiber and Epoxy resin for diffusing tip. The CDOFP was characterized to investigate the clinical feasibility and the results presented that the CDOFP may be used clinically by combining endoscopic method, such as ERCP or EUS, to treat cancer on pancreas and pancreaticobiliary duct.

10058-34, Session 9

Silver/polymer coated hollow glass waveguides for mid-IR transmission

Wesley Y. Kendall, James A. Harrington, Rutgers, The State Univ. of New Jersey (United States)

Hollow glass waveguides (HGWs) are optical fibers that are practicable for high-power transmission throughout the visible and infrared spectra. HGWs comprised of a silica capillary tube with inner coatings of silver and polymer thin films were developed for Er:YAG and CO2 laser transmission. Teflon™ AF, an amorphous fluoropolymer, was chosen due to its superior optical transmission at IR wavelengths as well as its low refractive index at mid-IR wavelengths. Additionally, poly(methyl methacrylate) (PMMA) and low-density polyethylene (LDPE) were selected for their chemical resistance, thermal stability, and ease of film creation. Silver was deposited onto the waveguide using established liquid chemistry techniques, while the polymer dielectric layers were added using a vacuum pump deposition method. FTIR spectroscopy was used to characterize the waveguides at determine the transmission of the guide at ? = 2.94 and 10.6 μm, as well as to determine the thickness of the polymer films. Loss properties were calculated using a cutback technique, measuring output from Er:YAG and CO2 lasers with fibers straight and at varying degrees of curvature. Furthermore, the modal outputs at both wavelengths were characterized using a spatial beam profiler.

10058-35, Session 9

Improvement of transmission properties of visible pilot beam for polymer-coated silver hollow fibers with acrylic silicone resin as buffer layer for sturdy structure

Katsumasa Iwai, Hiroyuki Takaku, Mitsunobu Miyagi, Sendai National College of Technology (Japan); Yi-Wei Shi, Xiao-Song Zhu, Fudan Univ. (China); Yuji Matsuura, Tohoku Univ. (Japan)

Flexible hollow fibers with 530-?-m-bore size were developed for infrared laser delivery. A sturdy hollow fibers were fabricated by liquid-phase coating technique. A silica glass capillary is used as the substrate. An acrylic silicone resin is used as a buffer layer and the buffer layer is firstly coated on the inner surface of the capillary to protect the glass tube from chemical damages due to the following silver plating process. A silver layer was inner-plated by using the conventional silver mirror-plating technique. To improve adhesion of catalyst to the buffer layer, a surface conditioner by condiriser solution has been introduced in the method of silver mirror-plating technique. We discuss improvement of transmission properties of sturdy polymer-coated silver hollow fibers for the Er:YAG laser and red pilot beam delivery.

10058-36, Session 9

Short- und long-term damage and annealing of improved UV-fibers using high-power light-source

Karl-Friedrich Klein, Philipp Raithel, Technische Hochschule Mittelhessen (Germany); Mathias Belz, World Precision Instruments (Germany); Christiane Jakob, TransMIT GmbH (Germany)

Multimode UV-fibers with core diameters from 70 to 600 μm diameter have been improved in respect to defect reduction due to fiber drawing and processing. Generated by D2-lamp light, UV-induced losses at 214 nm have been reached saturation levels below 0.5 dB, for 2 m long samples after 4 hours. However, the UV-induced loss is increasing slightly after 72 hours.

For applications in analytics or sensing, a plasma-based Laser-driven light source has been introduced as an alternative to D2-lamp. It has been shown, however, that the UV-defect concentration after 4 hours is lower although the spectral power density is significantly higher. Annealing effects due to vis- and IR-light portion of the new lamp has to be taken into account.

For the first time, we are concentrating on the short-term (< 5 min) effects of UV damaging and annealing in different UV-fibers due to different spectra; especially the light power above 400 nm will be blocked or at least significantly reduced. In addition, the long-term behavior with both lamps will be shown und discussed. The core diameter will be taken into considerations, too.

10058-37, Session PSun

Dual modal endoscopic cancer detection based on optical pH sensing and Raman spectroscopy

Soogeun Kim, ByungHyun Kim, Won Bum Sohn, Kyung Hee Univ. (Korea, Republic of); Kyung Min Byun, Soo Yeol Lee, Kyung Hee Univ. (Korea, Republic of) and Targeted Precision Treatment Research Ctr. (Korea, Republic of)

To discriminate between normal and cancerous tissue, a dual modal approach using Raman spectroscopy and pH sensor was designed and applied. Raman spectroscopy has demonstrated the possibility of using as diagnostic tool for the early detection of precancerous and cancerous lesions in vivo. It also can be used in identifying markers associated with malignant change. However, Raman spectra lack sufficient sensitivity due to very weak Raman signal or less distinctive spectral pattern. A dual modal approach could be one of the solutions to solve this issue. The value of extracellular pH in cancer tissue is lower than that in normal tissue due to increased lactic acid production, decreased interstitial fluid buffering and decreased perfusion. High sensitivity and specificity required for cancer diagnosis could be achieved by combining the chemical information from Raman spectrum with metabolic information from pH value. Raman spectra were acquired by using a fiber optic Raman probe, a cooled CCD camera connected to a spectrograph and 785 nm laser source. Tissue pH values were measured by a surface plasmon resonance sensor based on fiber optic. The diagnostic capability of pH-Raman dual modal method was evaluated using principal component analysis, linear discriminant analysis and cross-validation method with a leave-one-out approach. The obtained results showed that the pH-Raman dual modal approach can improve diagnostic capability for all the examined tissues, which lead to very high sensitivity and specificity. The proposed method for cancer detection is expected to be used in endoscopic diagnosis later.

10058-38, Session PSun

Radial tissue deformation during temperature-controlled photothermal treatment

Jinoh Bak, Hyun Wook Kang, Pukyong National Univ. (Korea, Republic of)

Esophageal stricture occurs in 7~23 % of patients with gastroesophageal reflux disease. However, the current treatments including stent therapy, balloon dilation, and bougienage involves limitations such as stent migration, formation of the new strictures, and snowplow effect. The purpose of the current study was to investigate the feasibility of structural expansion in tubular tissue ex vivo during temperature-monitored photothermal treatment with a diffusing applicator for esophageal stricture. Porcine liver was used as an ex vivo tissue sample for the current study. The glass tube was used to maintain a constant distance between the diffuser and tissue surface and to evaluate any variations in the luminal area after laser irradiation for potential stricture treatment. The 3D goniometer measurements confirmed that roughly isotropic distribution with less than 10% deviation from the average angular intensity over 2ϖ (i.e., 0.86±0.09 in arbitrary unit) from the diffusing applicator. The 30-s irradiation increased the tissue temperature up to 72.5 °C, which was close to the temperature for irreversible thermal denaturation (i.e., 65~75 °C). The temperature became saturated at 70 °C after 58-s irradiation (i.e. steady-state error = ± 0.4 °C). The rest of the irradiation times longer than 5 s presented area expansion index of 1.00 ± 0.04, signifying that irreversible tissue denaturation permanently deformed the lumen structure in a circular shape and maintained the equivalent luminal area to that of the glass tube. Application of a temperature feedback controller for photothermal treatment with the diffusing applicator can regulate the degree of thermal denaturation to effectively treat esophageal stricture in tubular tissue.

10058-39, Session PSun

Investigation into surface interaction between the contact lens and the upper eyelid margin using optical coherence tomography

Rachel Morecroft, The Univ. of Sheffield (United Kingdom); Peter Toomey, Sheffield Teaching Hospitals NHS

Foundation Trust (United Kingdom); John E. Goff, Peter Mylon, Matt J. Carré, Stephen J. Matcher, Roger Lewis, Raman Maiti, The Univ. of Sheffield (United Kingdom)

There are over 100 million contact lens users worldwide and 70% of the users report some level of discomfort, such as dryness and irritation. Anterior segment optical coherence tomography (OCT), with high resolution imaging of 10 μm (axial) and 30 μm (transverse) and 30,000 A-scans per second, was used to quantify the morphological differences (abrasion, surface smoothness and skin layer thickness) of the upper eyelid Lid Wiper (LW) between contact lens users and non-contact lens users. The study of the interaction between a contact lens and the eyelid is important for making future contact lenses more comfortable.

10058-40, Session PSun

Cell counting system by using single fiber interferometer

Seung Seok Lee, Joo Ha Kim, Chosun Univ. (Korea, Republic of); Woosub Song, Korea Photonics Technology Institute (Korea, Republic of); Eun-Seo Choi, Chosun Univ. (Korea, Republic of)

We proposed cell-counting method based on optical fiber interferometer and demonstrated the performance of the proposed method. Cell counting means the counting or quantification of individual cells and its application ranges from the life science to medical diagnosis. As a conventional approach for cell counting, flow cytometry is employed. Because the flow cytometry uses bulk and expensive equipment, it was not used for only purpose of cell counting. When image analysis method is exploited, the limited field of view obtained by microscope is used for cell counting. Therefore, time consuming for whole counting of cells is to be solved. The proposed method utilized single fiber and spectrometer. Light beam of the light source having broad spectral bandwidth over 100 nm at 850 nm central wavelength is irradiated to a flow channel through single fiber from top and bottom. Before passing a cell, optical path is one across beam diameter, where beam size covers the width of the flow channel. Two different optical paths are made only when the cell is passing though the flow channel across the beam area. The difference of optical path lengths in the beam area induces interference depending on optical thickness of the cell. By measuring a series of interferences, the number of cells can be analyzed properly. The proposed system can be implemented without any expensive and perform cell counting in the absence of complex algorithm. Therefore, it can be a good alternative to the reported cell-counting methods.

10058-41, Session PSun

Colourimetric chemosensor for copper, fluoride, and cyanide ions: Its application in molecular logic gate

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The design and development of selective optical sensors and receptors for anions and cations have gained considerable attentions, due to its essential role in many areas such as medicinal biological, environmental chemistry and catalysis. The anthracene based dithiosemicarbarbazone was synthesized and characterized, and which was used as a selective optical and naked eye chemosensor towards copper, fluoride and cyanide ions. The presence of fluoride and cyanide ion show a new broad band at 519 nm, and for copper ion the broadness is at 610 nm. The receptor shows higher binding affinity towards copper ions than anions, and the binding constant for copper ion was found to be 2.83x107 M-1. The sensing process was further checked with emission spectra. The presence of copper quenched the fluorescence intensity and presence of fluoride and cyanide ions enhance the fluorescence intensity. The chemosensor shows fluorescence ON-OFF behavior with Read-Erase-Write-Read property when it treats with an alternative addition of Ca2+ and F-, which results the INHIBIT logic gate at the molecular level using F- and Ca2+ as chemical inputs and the fluorescence intensity signal as out puts. While comparing the optical properties of the receptor with the presence of individual ions and a combination of the ions, we developed a molecular logic gate capable of mimicking the functions of AND, OR, NAND and XOR logic gates with the input of Cu2+, CN- and F- ions.

10058-42, Session PSun

Effects of handgrip exercise over radial arterial pulse pressure waveform

Shweta Pant, Sharath Umesh, Sundarrajan Asokan, Indian Institute of Science (India)

Physical exercises are known to elicit the cardiovascular functions. Radial arterial pulse pressure waveform (RAPPW) is an indicator of the cardiovascular status of the subject. The present study reports about the RAPPW variations at various stages during the performance of handgrip exercise. The RAPPW is acquired using a fiber Bragg grating probe, which has the ability to dynamically acquire the arterial pulse pressure. The pulsating radial artery strikes the silicone diaphragm creating strain variations over it, which are acquired by the fiber Bragg Grating sensor adhered on it. Pulse wave analysis in the amplitude, time and frequency domains are carried out to evaluate vital cardiovascular functions like heart rate, peripheral augmentation index, left ventricular ejection time and ejection duration index. Four different stages of handgrip exercise are considered for pulse wave analysis. Specific changes in the cardiovascular functions are observed after the performance of the exercise by the subject at different levels of exertion. Comparison of the RAPPW obtained at different exertion levels are carried out with respect to the pulse pressure curve obtained along with the cardiovascular functions evaluated. This study is intended to assess the effect of handgrip exercise over the RAPPW and further it may be extended to evaluate the utility of physical exercises in order to improve the cardiovascular status of the subject.

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10059-1, Session 1

Validation of new technologies for enumeration of rare circulating cells in vivo *(Invited Paper)*

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Circulating cells (CCs) in the bloodstream play an important role in many diseases. As such, there has been significant interest in development of new pre-clinical tools for enumeration of single CCs in vivo that do not require drawing and analysis of blood samples. Our group has specifically focused on the problem of enumeration of rare CCs, for study of disease processes (such as cancer metastasis) involving CCs at concentrations below 100 cells/mL blood.

To achieve this, we recently developed and performed 'proof-of-principle' validation of two new techniques that optically sample relatively large volumes of circulating blood. Briefly these are, i) Diffuse Fluorescence Flow Cytometry (DFFC), which uses diffuse photons to interrogate a large region of a mouse limb in diffuse reflectance (DR) or diffuse transmittance (DT) geometry, and, ii) 'Computer Vision In Vivo Flow Cytometry" (CV-IVFC), where the mouse ear is fluorescently-imaged with a widefield macroscope.

In this presentation, we discuss our work in characterizing the performance of these technologies. Specifically, we developed a new instrument that allows concurrent CV-IVFC, DR-DFFC and DT-DFFC on the same animal. We tested this system with fluorescently-labeled circulating Multiple Myeloma cells mice. We directly compared the performance against "true" cell counts obtained by drawing blood samples from the mice, and showed that each method allows enumeration of cells below 100 cells/mL in a 10 minute scan. We anticipate that our technology will have use in many applications involving rare CC populations.

10059-2, Session 1

Simultaneous and co-localized acousto-optic measurements of blood flow and oxygenation

Michal Balberg, Holon Institute of Technology (Israel); Sergio Fantini, Tufts Univ. (United States)

In order to assess local changes in the cerebral metabolic rate of oxygen (CMRO2), both oxygen saturation (i.e. spectral information) and blood flow within the measured brain tissue need to be determined simultaneously. Acousto –optics enables to locally measure changes in blood flow and in the color of hemoglobin within a specific volume by modulating coherent light as it diffusely propagates through the focus of an ultrasound beam.

We demonstrate, in a tissue phantom, that local changes in flow rate in a specific layer can be extracted simultaneously and independently of changes in the color of the phantom layer. A phantom model constructed of different layers of micro-channels at different depths, embedded within an optically scattering but acoustically transparent material, is illuminated with coherent light at 785nm and 852nm sequentially (coherence length>1m), while an ultrasound (central frequency 1MHz) wave is variably focused on different layers of micro-channels. The flow rate is varied using a syringe pump, while the spectral information is varied using different concentrations of a dye. The temporal broadening (Doppler) of the detected light at each focusing depth is measured concurrently with the spatial decay of the amplitude of the modulated signal at the acoustic frequency as a function of the focal depth of the ultrasound, for each wavelength of light. A two dimensional map of the color and flow rate at each depth will be presented.

10059-3, Session 1

Diffuse speckle contrast analysis with novel fiber-lens detection

Chaebeom Yeo, Cheol Song, DGIST (Korea, Republic of)

To date, various minimally-invasive or noninvasive blood flow measurement systems have been developed, including fMRI, LDF, and DCS. Recently, diffuse speckle contrast analysis (DSCA) is demonstrated in relatively deep tissues as noninvasive in-vivo blood flow monitoring system. The main components are a laser source (785nm) and a camera. The DSCA estimates blood flow index (BFI) by calculating a correlation of speckle patterns caused by moving scatters in the blood. One of the system configurations is lens based DSCA, although it has simple setup, the defocusing caused by sample's motion artifact can affect negative image quality. Another configuration, fiber-optics based DSCA, can reduce the motion artifact and measure simultaneous blood flow on various positions with multi-channel probes. However, to closely place the optical detection probes at the camera sensor for better signal quality, the protection glass in front of the camera sensor has to be removed., The camera gradually will be contaminated and not reused for different purposes. Without removing the glass, the beam from the fibers cannot be detected and analyzed due to the beam divergence. Here, we present a novel fiber-lens combined DSCA which can solve it. The beam of the fiber tip can be focused on the camera sensor through a biconvex lens (Magnitude=1). This system has been applied to cerebral blood flow (CBF) monitoring of rats during middle cerebral artery occlusion surgery. As a result, the system showed relative changes of the blood flow during the arterial perfusion periods.

10059-4, Session 1

Clinical applications of high-speed blood flow measurements with diffuse correlation spectroscopy

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Diffuse Correlation Spectroscopy (DCS) is an increasingly popular non-invasive optical technique to clinically measure deep tissue blood flow, albeit at slow measurement rates of 0.5-1 Hz. We recently reported the development of a new 'fast' DCS instrument that continuously measures blood flow at 50-100 Hz (simultaneously from 8 channels), using conventional DCS sources/detectors, and optimized software computations. A particularly interesting result was our ability to optically record pulsatile micro-vascular blood flow waveforms, and therein readily identify high frequency features such as the dicrotic notch. Here, we showcase the utility and potential of high-speed measurements of blood flow (and arterial blood pressure) in a few clinical applications. First, we employ the fast-DCS instrumentation to measure cerebral autoregulation (CVAR) dynamics. Cerebral autoregulation refers to the mechanism by which cerebral blood flow (CBF) is maintained during fluctuations in blood pressure; CVAR is impaired in the injured brain. We derive an index of autoregulation by measuring the rates of decrease (and recovery) of blood flow and blood pressure following a sudden, induced change in systemic blood pressure (e.g., bilateral thigh cuff deflation). Our pilot experiments in healthy volunteers show that DCS measured rates of micro-vascular regulation are higher than conventional large vessel regulatory metrics (e.g., measured with transcranial Doppler ultrasound). Second, we utilized pulsatile blood

flow oscillations in cerebral arteries to estimate the critical closing pressure (CrCP), i.e., the arterial blood pressure at which CBF approaches zero. Pilot experiments in healthy subjects show good agreement between CrCP measured with DCS and transcranial Doppler ultrasound.

10059-5, Session 1

Progress on time-domain diffuse correlation spectroscopy

Davide Tamborini, Bernhard B. Zimmerman, Jason Sutin, Kuan-Cheng Wu, David A. Boas, Maria A. Franceschini, Athinoula A. Martinos Ctr. for Biomedical Imaging, Harvard Medical School (United States)

Time-Domain Diffuse Correlation Spectroscopy (TD-DCS) is a novel method that merges diffuse correlation spectroscopy (DCS) and time-domain near-infrared spectroscopy (TD-NIRS) in a single device. This allows us, with a single measurement, to acquire the TD-NIRS temporal point spread function (TPSF) to quantify tissue optical properties as well as the DCS autocorrelation function to quantify blood flow index (BFi). In addition, it enables the application of time-gated strategies to the time-resolved autocorrelation functions, allowing differentiation between short and long photon paths and the determination of BFi at different depths.

We will present the progress made in moving the TD-DCS system to a clinical setting and the development of several custom electronics used to achieve this goal. One custom component we developed is a picosecond pulsed laser source with high peak power able to provide 150 ps full-width half-maximum pulses at a 150 MHz repetition rate with a sufficient coherence length for DCS measurements. In addition, we utilized the new class of red-enhanced single photon avalanche diode (SPAD) detectors which combine high-temporal resolution (50 ps FWHM) with high photon detection efficiency (25%) in the near infrared range. We also designed a custom FPGA-based board to host time-correlated single photon counting cards used for measuring the time of flight of individual photons, as well as pair this information with the absolute photon arrival times, measured by a counter inside the FPGA that is locked to the laser frequency. Finally we will present the characterization and validation studies in tissue-like phantoms and in vivo experiments.

10059-6, Session 1

Simultaneous estimation of absolute blood oxygenation and flow index by multi-color multi-distance diffuse correlation spectroscopy

Parisa Farzam, Davide Tamborini, Bernhard B. Zimmermann, Kuan-Cheng Wu, Jason Sutin, David A. Boas, Maria Angela Franceschini, Athinoula A. Martinos Ctr. for Biomedical Imaging, Harvard Medical School (United States)

Diffuse correlation spectroscopy (DCS) measures microvascular blood flow index (BFi). Since DCS analysis requires prior information of absorption (?a) and reduced scattering (?s?) coefficients, it is customary to deploy diffuse optical spectroscopy in tandem with DCS NIRS in hybrid devices.1

Towards the development of a stand-alone DCS system, it was demonstrated that it is possible to uniquely determine ?a, ?s?, and BFi from multi-distance DCS for short-separations (r < 5 mm)2. To increase the sensitivity to deeper tissue, we propose a novel approach that employs multi-wavelengths multi-distance DCS to uniquely separate the optical properties and BFi. While measuring slope of intensity decay over distances results in ?a.?s?, decay of the autocorrelation function at three or more wavelengths provide the remaining independent measurements to uniquely determine all the parameters of interest. We have validated the method's robustness by numerical simulations.

Following those promising results, we have built a state-of-the-art multi-wavelength, multi-distance system. This system exploits novel long-coherence-length-lasers controlled by a custom laser driver board allowing fast multiplexing of three colors. The diffused light is collected from different separations by single-photon APDs. The FPGA-based correlator board records the single-photon arrival time, enabling the tuning of repetition rate in post-processing. In summary, we demonstrate the new generation of DCS with an extensive improvement in both instrumentation and data analysis that sets the stage for a new range of clinical applications.

Acknowledgements: Grants R01GM116177 and R21NS093259.

1. D. A. Boas et al., Phys. Rev. Lett. 75, 1855 (1995).

2. P. Farzam et al., J. Biomed. Opt., 20, 55001 (2015).

10059-7, Session 2

Multispectral compressed single pixel imaging in the spatial frequency domain

Mohammad Torabzadeh, Beckman Laser Institute and Medical Clinic (United States); Il-Yong Park, Dankook Univ. (Korea, Republic of); Anthony J. Durkin, Bruce J. Tromberg, Beckman Laser Institute and Medical Clinic (United States)

Single pixel imaging based on sparse sampling is an evolving technology in biomedical imaging. This approach replaces 2-dimensional arrays of pixels with single pixel photodetectors, making the technique relatively inexpensive in terms of detector cost. Applying compressed sensing algorithms to this approach allows for reducing the number of sparse samples, thus increasing the acquisition speed. In addition, the high temporal bandwidth of a single-element photodetector enables encoding a changing temporal intensity modulation on the output power spectrum of the illumination source. This allows one to employ light sources with different wavelengths without substantial increase in acquisition time. Our group has incorporated the compressed single pixel imaging technique into a Spatial Frequency Domain Imaging (SFDI) setup. SFDI is non-invasive wide-field spectral imaging technique which can be used to produce quantitative maps of tissue optical properties and chromophore concentrations. For this purpose, spatially modulated illumination is projected onto the sample and the remitted reflectance is detected with a camera. Taken at phase shifted illumination patterns, the demodulated reflectance image, in combination with a model of light transport, produces absorption and scattering maps from a region of interest. Here, we present the design of a SFDI setup that employs a single-pixel camera on the detection side and a three-wavelength LED source. The compressed SFDI setup extracts absorption and reduced scattering coefficients of a tissue phantom within 6% and 1% of the known values, respectively. This system computes tissue properties at 12 frames per second with 64?64 pixel resolution in an in-vivo pressure cuff occlusion experiment.

10059-8, Session 2

Hyperspectral spatial frequency domain imaging from 680-1,300 nm for improved estimation of tissue water and lipid concentrations

Yanyu Zhao, Kavon Karrobi, Boston Univ. (United States); John P. Dumas, Mark C. Pierce, Rutgers, The State Univ. of New Jersey (United States); Darren M. Roblyer, Boston Univ. (United States)

Spatial frequency domain imaging (SFDI) is an emerging modality for non-invasive, wide-field quantification of tissue optical properties. To-date, most

SFDI systems have used visible and/or near-infrared (NIR) light to determine the concentrations of tissue chromophores with distinct absorption features in these spectral regions, such as oxy- and deoxy-hemoglobin. Other biologically relevant chromophores such as lipid and water exhibit strong absorption bands at longer wavelengths, beyond 1,000 nm. To extract concentration values for all of these chromophores, SFDI instrumentation operating from the NIR to the short-wave infrared (SWIR) region is required.

We have assembled a benchtop SFDI platform which targets chromophore absorption peaks at 680nm and 850nm for hemodynamic parameters, 970nm for water, and 1,210nm for lipids. A femtosecond laser with output tunable across this spectral range illuminates a digital micromirror device for projection of structured intensity patterns onto a sample. Diffuse optical reflectance is captured by either a wideband germanium CMOS camera covering 400–1,600nm, or a co-registered combination of sCMOS (300–1,000nm) and InGaAs cameras (1,000–1,700nm).

After demodulation of raw diffuse reflectance images and correction for instrument-specific factors against a calibrated phantom, absorption and reduced scattering coefficients are calculated using a Monte Carlo model-based look-up table. Initial testing in solid tissue simulating phantoms demonstrated optical properties extractions within 12% of a gold standard. This presentation will report our findings on the accuracy of hemoglobin, lipid and water concentrations measurements using conventional NIR wavelengths versus measurements using the extended NIR-SWIR wavelength range.

10059-9, Session 2

Structured light scatteroscopy through a rigid endoscope provides unique real-time contrast based on tissue ultrastructure

Jonathan T. Elliott, David M. McClatchy III, Mackenzie L. Carlson, Thomas Usherwood, Venkataramanan Krishnaswamy, Keith D. Paulsen, Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

High frequency spatially modulated light provides scatter-dominant contrast that is sensitive to microscopic changes in bulk tissue morphology—a clinically useful source of image contrast. Wide-field structured light scatteroscopy has shown superb sensitivity to tissue scatter amplitude and phase-function, and while previous groups have demonstrated the possibility of spatial frequency domain imaging in flexible endoscopes, the finite number and size of fiber bundles in these systems impose an upper limit on the scatter-weighted high-frequency information that can be transmitted. Here we characterize a lens-array based rigid stereoendoscope system which uses one side to transmit structured light from digital light projector to the tissue surface and the other side to image the surface with a color camera. High spatial-frequency color images are processed, demodulated and displayed in real time next to a standard color white-light image of the tissue. The capabilities of the system are demonstrated using test targets, intralipid phantoms and ex vivo animal tissue. The ability to visualize scatter-based contrast in real time could allow the surgeon to more easily integrate new information and assess areas of suspicion not obvious on color endoscopy alone.

10059-10, Session 2

Enhanced scatter contrast color imaging of tissue: Methods for comparing high spatial frequency domain and cross-polarization scatter images

Mackenzie L. Carlson, David M. McClatchy III, Brian W. Pogue, Jonathan T. Elliott, Stephen C. Kanick, Thayer School of Engineering at Dartmouth (United States); Keith D Paulsen, Thayer School of Engineering at Dartmouth College (United States) and Norris Cotton Cancer Center at Dartmouth Hitchcock Medical Center (United States); Jason R Gunn, Thayer School of Engineering at Dartmouth College (United States)

Unique and enhanced contrast in tissues based on microstructure and composition is demonstrated by a new imaging approach, high spatial frequency RGB modulated imaging. Both cross-polarized imaging and high spatial frequency RGB modulated imaging techniques are used to differentiate tissues based on scattering properties. In comparing these two modalities, it was demonstrated that high spatial frequency RGB modulated imaging provides improved contrast over cross-polarized imaging. The modulated imaging method is more sensitive to changes in intralipid levels independent of the color of the sample and has demonstrated a wider dynamic range of contrast in tissues with higher intralipid concentration. Additionally, scattering signal intensity increases linearly with intralipid concentration, whereas with cross-polarized imaging, scattering signal intensity decreases exponentially as intralipid concentration increases. This method of high frequency RGB modulated imaging could affect diagnostic imaging of skin, where cross-polarized imaging is the current state-of-the-art modality.

10059-11, Session 3

Fiber-based multiphoton probe for z-resolved autofluorescence spectroscopy of living tissues

Pierre Leclerc, Charles-Henri Hage, Flavien Befarra, Marc Fabert, Julien Brevier, XLIM Institut de Recherche (France) and Univ. de Limoges (France); Rémi Habert, Flavie Braud, Alexandre Kudlinski, Lab. de Physique des Lasers, Atomes et Molécules UMR-CNRS 8523 (France); Sergei G. Kruglik, Christine Vever-Bizet, Geneviève Bourg-Heckly, Univ. Pierre et Marie Curie (France); Luc Thiberville M.D., Rouen Univ. Hospital (France); Anne Druilhe, Frederic Louradour, Univ. de Limoges (France)

A multiphoton fiber-optic probe (O.D. < 2.4 mm), made of a 5-meter long custom-made double-clad photonic crystal fiber, featuring tunable femtosecond excitation, native 2-photon excited fluorescence (2PEF) and second harmonic generation (SHG) spectroscopy, and depth resolved z-scanning was developed. The system enables to probe tissues up to 500 microns depth below the surface and to study the evolution of the optical spectral signature of endogenous cellular and tissular components, layer after layer, with an axial resolution of 10 microns.

The development of epithelial cancers is characterized by changes in cellular metabolism and early modifications of the extra-cellular matrix (ECM) fibrillar collagen and elastin network. The capability of performing z-depth optical sectioning, together with excitation-wavelength-tunability, makes possible to separate the metabolic contribution of the epithelium (through cellular NAD(P)H and flavins co-enzymes 2PEFs) from the tissue ECM structural contribution (through elastin 2PEF and collagen SHG).

In order to assess the diagnosis potential of our probe, several studies were performed on healthy and cancerous human lung cell lines and ex vivo lung samples. In cells, we measured the cellular optical redox-ratio, based on the analysis of NAD(P)H and flavins spectra, which is an indicator of the cellular metabolic state. In the lung samples, associated spectra of the ECM collagen (through SHG) and elastin (through 2PEF) emissions were simultaneously measured in order to follow the structural modifications of the ECM associated with neoplastic transformation. Complementarily, ongoing experiment on other animal models (e.g. mouse kidney fibrosis) will be also presented.

10059-12, Session 3

System design and performance evaluation of a fluorescence laminar optical tomography scanner for brain studies

Mahya Sheikhzadeh, Mehdi Azimipour, Ramin Pashaie, Univ. of Wisconsin-Milwaukee (United States)

Implementation of new fluorescent reporter technologies for monitoring molecular and cellular processes non-invasively is highly desirable. The tomography technique presented here, the Fluorescence Laminar Optical Tomography (FLOT), enables us to derive quantitative information clarifying the three-dimensional distribution of a fluorescent probe or protein concentration in the brain of small rodents. The system is specifically designed for functional imaging of superficial tissue, with applications in different biomedical research areas, including neuroscience experiments where fluorescence imaging and molecular genetic methods are used to study the dynamics of the brain circuitries. In this design, a line-shaped collimated laser beam is scanned over the surface of tissue, using a set of galvanometer mirrors, to excite fluorophores within the tissue. A highly sensitive EMCCD camera records the emission fluorescence signal through the imaging components of the system. The forward model for the image reconstruction is based on Monte Carlo simulation for light propagation in inhomogeneous tissue. This model is used to produce sensitivity matrices for every source-detector separations. The inverse problem is solved using an iterative reconstruction method, the simultaneous algebraic reconstruction technique (SART). The setup was tested using silicon-based microchannel phantoms with the optical properties close to brain tissue, and ultimately by scanning the brain tissue in-vivo. The FLOT scanner has shown promising results in imaging superficial areas up to 2mm deep from the surface, with the resolution of ~200μm. Details of the design of the hardware for the scanner and reconstruction algorithms are discussed in the paper and several experimental results are presented.

10059-13, Session 3

Monitoring temporal microstructural variations of skeletal muscle tissues by multispectral Mueller matrix polarimetry

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Mueller matrix polarimetry is a powerful tool for detecting microscopic structures, therefore can be used to monitor physiological changes of tissue samples. Meanwhile, spectral features of scattered light can also provide abundant microstructural information of tissues. In this paper, we take the 2D multispectral backscattering Mueller matrix images of bovine and porcine skeletal muscle tissues, and analyze their temporal variation behavior using quantitative Mueller matrix parameters. The 2D images of the Mueller matrix elements are reduced to the multispectral frequency distribution histograms (mFDHs) and their corresponding central moments to reveal the dominant structural features of the muscle samples quantitatively. In addition, a group of new parameters based on mFDHs are proposed to characterize the microstructural variations during the proteolysis process of the muscle tissue samples. The experimental results indicate that the new mFDH based parameters can be used to judge different physiological stages for both bovine and porcine muscle tissues and distinguish the two kinds of samples. Contrast mechanism of the multispectral Mueller matrix imaging for muscle tissues is backed up by Monte Carlo (MC) simulations based on the sphere-cylinder birefringence model, which reveals the relationship between the mFDHs based parameters and the microstructures of the muscle samples. The results presented in this work show that combining with the multispectral technique, the Mueller matrix polarimetry and FDH analysis can monitor the microstructural variation features of both bovine and porcine muscle tissues. The techniques may be used for quantitative assessment of meat qualities in food industry.

10059-14, Session 4

Miniaturized and robust Ultrasound guided diffuse optical tomography system for breast cancer detection

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According to the World Health Organization, breast cancer is the most common cancer among women worldwide, claiming the lives of hundreds of thousands of women each year. Near infrared diffuse optical tomography (DOT) has demonstrated a great potential as an adjunct modality for differentiation of malignant and benign breast lesions and for monitoring treatment response of patients with locally advanced breast cancers. The path toward commercialization of DOT techniques depends upon the improvement of robustness and user-friendliness of this technique in hardware and software. In the past, our group have developed three frequency domain prototype systems which were used in several clinical studies. In this study we introduce our newly developed US-guided DOT system which has been improved in terms of size, robustness and user friendliness by several custom electronic and mechanical design. A new and robust probe designed to reduce preparation time in clinical process. The processing procedure, data selection and user interface software have been improved. With all these improvements, our new system is more robust and accurate which is one step closer to commercialization and wide use of this technology in clinical settings. This system can be used by minimally trained user in the clinical settings with robust performance. The system performance has been tested in the phantom experiment and initial results are demonstrated in this study. We are currently moving toward use of this system in clinical setting for patients with breast cancer.

10059-15, Session 4

Intensity-modulated wavelength-swept laser-based diffuse optical spectroscopy to monitor blood oxygenation

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Diffuse optical spectroscopy is a non-invasive tissue imaging method using photon diffusion in media. Because human tissue consists of various chromophores, such as hemoglobin, lipid and water, and each chromophore has different absorption spectrum, multi-wavelength light sources are suggested for diffuse optical spectroscopy. Scattering coefficient is another significant factor that must be considered to identify composition of the tissue. In order to extract a scattering coefficient from total absorbance, intensity-modulated light sources are commonly used to get a phase delay between input and detected signals. Although it is necessity, it is very difficult to construct a light source which has both wide spectrum and modulated intensity. For this reason, most diffuse optical spectroscopy systems have adopted a white light source or an array of laser diodes.

In this paper, we proposed novel intensity-modulated wavelength-swept laser for diffuse optical spectroscopy system. The wavelength-swept laser consists of semiconductor optical amplifier with gain spectrum around NIR region (780 ~ 820 nm) and acousto-optic tunable filter to select a lasing wavelength within the gain region. Output wavelength is linearly changed by controlling the RF frequency into the acousto-optic tunable filter. To modulate the intensity of wavelength-swept laser with 70 MHz, active

mode locking technique is implemented to match the free spectral range of cavity and the applied signal into the optical gain. By using the verified performance of laser source, various experiment with tissue mimicking phantom and in-vivo blood oxygenation test are simply implemented for identifying the modality of constructed diffuse optical spectroscopy system.

10059-16, Session 4

Improved screening of esophageal cancer by combining optical coherence tomography and single fiber reflectance spectroscopy

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Esophageal adenocarcinoma has a poor prognosis when detected at a symptomatic stage. Patients with Barrett's esophagus are at an increased risk and therefore undergo regular endoscopic surveillance. When detected early, endoscopic treatment is possible, thereby avoiding extensive surgery. The current surveillance method is endoscopic inspection followed by quadrantic random biopsies. However, lesions are often missed due to subtle appearance upon endoscopy and sampling errors. Therefore, new technologies for improving risk stratification for Barrett's esophagus patients are warranted. Combining endoscopy with optical techniques has the promise to be a simple and cost effective diagnostic tool for detection of esophageal adenocarcinoma. We are therefore studying the combination of endoscopy with both Optical Coherence Tomography (OCT) and Single Fiber Reflectance Spectroscopy (SFR).

We will include 60 patients: 30 with non-neoplastic and 30 with neoplastic Barrett's Esophagus. Both techniques are fiber-based and introduced simultaneously through the endoscope. Measurements will be performed in normal appearing Barrett's segments, dysplastic lesions (if present), squamous epithelium and buccal (oral) epithelium. With OCT we determine the attenuation coefficient of the sampled tissue at 1310 nm. With SFR we investigate absorbers, the reduced scattering coefficient and ? at 400-900 nm. In addition to the regular random biopsy protocol, two biopsies will be taken from a normal appearing Barrett's segment and two from a dysplastic lesion (if present). Co-localization of the optical measurements and targeted biopsies is ensured by placing electrocoagulation markers.

Patient measurements have already started. First results comparing the optical measurements to histopathology will be presented at the conference.

10059-17, Session 4

Diffuse optical tomography based on time-resolved compressive sensing

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Diffuse Optical Tomography (DOT) aims to quantitatively reconstruct absorbing and scattering properties of inclusions within in vivo organisms. The measurement scheme consists of illuminating the sample and detecting the diffused light exiting from it. By solving the inverse problem, the optical parameters of each sample voxel can be quantitatively reconstructed. Beyond spatial sampling, multiple views, spectral and temporal information, are fundamental to improve the tomographic capability, hence, DOT can be described as a highly multidimensional problem generating a huge data set with long acquisition/computational times.

Biological tissue behaves as a low pass filter in the spatial frequency domain, hence compressive sensing approaches are extremely useful to reduce the data set while preserving the information content. This paradigm leads to exploit both a patterned illumination and detection; the latter following the single-pixel camera scheme. A compressive approach, beyond a reduction of the data set, brings other remarkable advantages such as wide field illumination, higher performances of single detector (respect to parallel detector) and a reduction of cost and complexity of the system.

In this work, a multiple-view time-domain compressed sensing (illumination/detection) DOT system is presented and experimentally validated on non-planar tissue-mimicking phantoms containing both absorbing and scattering inclusions demonstrating a state of the art reconstruction quality. Moreover, the dependence of system imaging capability with the choice of illumination/detection patterns, number of views and temporal gates will be discussed. This aspect is fundamental to optimize both imaging and tomographic capability of the proposed system, while preserving the information content.

10059-18, Session 4

Lightweight high-density diffuse optical tomography using sCMOS detection

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Though optical neuroimaging is gaining momentum, widespread adoption has been restricted by the tradeoff between cap wearability and brain coverage. To maintain high-density imaging arrays and image quality that is comparable to fMRI, increased coverage requires more fibers and larger imaging consoles. However these changes drastically reduce the wearability of the imaging cap and the portability of the entire system. The primary obstacle for optimizing wearability is cap weight, which is largely determined by the size of the detection fibers: larger fibers collect more light and provide better signal-to-noise. Here we report on a design that leverages the exquisite sensitivity of scientific CMOS cameras, along with noise reduction algorithms to use fibers with ~30x smaller cross-sectional area than current high-density diffuse optical tomography (HD-DOT) systems. We have created a Super-Pixel sCMOS DOT (SP-DOT) system that uses 200um diameter source and detector fibers, with a lightweight, low-profile, and wearable cap design. Further, we developed a Super-Pixel algorithm with pixel binning and electronic noise subtraction to provide high dynamic range (>10^5), high frame rate (>6Hz), and low noise-equivalent-power (< 9fW/√Hz) comparable to previous HD-DOT systems. To assess system performance, we performed retinotopic mapping of visual cortex by having subjects view an angularly sweeping reversing checkerboard wedge rotating around the center of the screen. The sCMOS-based SP-DOT system design provides a novel approach to changing the weight/coverage tradeoff for the better, and the successful retinotopic mapping suggests that the signal-to-noise will allow SP-DOT to address a greater variety of applications.

10059-19, Session 4

The mechanism of tissue optical properties change induced by tissue deformation

Xu U. Zhang, Henricus J. C. M. Sterenborg, Dirk J. Faber, Ton G. van Leeuwen, Academisch Medisch Centrum (Netherlands)

Many forms of elastic scattering spectroscopy require direct contact between the fiber optical probe and tissue. Pressure application from the contact will induce tissue deformation which will eventually change tissue composition and structures, so we hypothesize it is tissue deformation that leads to body water displacement that influences tissue optical properties. To verify this hypothesis, we performed separate in vivo visible and near-infrared Single Fiber Reflectance spectroscopy (SFR) measurements on one volunteer's outer forearm skin with varied tissue deformation. VIS and NIR SFR allowed us to quantify blood volume fraction and water volume fraction respectively. We used a previously published, semi-empirical Single Fiber Reflectance spectroscopy model to extract the reduced scattering coefficient, blood volume fraction and water volume fraction from the reflectance spectra.

The VIS SFR data shows a blood volume decrease and reduced scattering coefficient increase as the indentation increases; similarly, the NIR data shows a water volume fraction decrease and reduced scattering coefficient increase as the indentation increases. A correlation between blood volume fraction decrease and water volume fraction decrease is observed when indentation increases from 0 to 9 mm; when indentation increases from 9mm to 12.5 mm, blood volume fraction decrease stops, while water volume fraction decreases further. A linear correlation was observed between water volume fraction and the reduced scattering coefficient. This indicates water volume displacement generated by tissue deformation changes tissue composition within the sampling volume such as an increase of high scattering collagen, which results in the reduced scattering coefficient increase.

10059-62, Session PMon

Feasibility study of spatial frequency domain imaging using a handheld miniaturized projector and rigid endoscope

Sreyankar Nandy, Washington Univ. in St. Louis (United States); Mohsen Erfanzadeh, Feifei Zhou, Univ. of Connecticut (United States); Quing Zhu, Washington Univ. in St. Louis (United States)

The initial feasibility of a spatial frequency domain imaging system was studied consisting of a hand held miniaturized projector and a rigid endoscope. Three wavelengths and two spatial frequencies were used for imaging. The system was calibrated using tissue mimicking phantoms. In vivo imaging was performed on five live mouse tumor models, and the absorption, scattering, hemoglobin oxygen saturation was measured. The initial promising results indicate that the spatial frequency domain imaging has the potential to be translated from the bench to the bedside in the operating room and can be a very useful tool for quantitative wide field tissue evaluation during minimally invasive image guided surgery.

10059-63, Session PMon

Single snapshot determination of absorption coefficient by multi-frequency MTF characterization in spatial frequency domain

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We present a novel approach for single snapshot determination of absorption coefficient based on multi-frequency modulation transfer function (MTF) characterization from measurement in spatial frequency domain. The adopted Fourier transform domain analysis enables simultaneous extraction of the applied frequencies and excellent reduction of noise. Simulations were conducted that respectively verified the feasibility of the MTF based approach and the performance of single snapshot determination of absorption coefficient using multi-frequency measurements. Phantom experiments without reference measurement demonstrated the high accuracy of absolute absorption coefficient determination with a maximum reconstruction error of 0.002 mm-1.

10059-64, Session PMon

Laser speckle imaging contrast enhancement in the presence of microbubbles

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A quantification of treatment effects needs a monitoring of the vessel changes over time in most skin diseases. Screening the location of blocked vessels can help planning for treatment towards the exact spot of perfusion within tissue beds in vivo. Laser speckle imaging (LSI) recently has been used to monitor blood perfusion within tissue and skin for shallow penetration depths. In this current study, micrometer-sized microbubbles were used as intravascular LSI contrast agents. The LSI was investigated as a method to detect stagnant microvessels (no blood flow) compared to flowing microvascular vessel when using microbubbles as exogenous contrast agents.

Microfluidic scattering phantom devices with one 300 micron channel were fabricated to model vascular blood flow via a syringe pump. A He–Ne laser (? = 632.8 nm, 3mW) beam was expanded to illuminate a 0.8 mm ? 1 mm area of microfluidic channel, which was imaged onto a CCD camera. The channel was filled with human blood and laser speckle images were evaluated under varying flow rates. LSI contrasts were then compared for with and without the presence of microbubble conditions for different blood flow rates. The dependency of LSI contrast on blood flow was also investigated and indicated contrast enhancement for faster blood flow when microbubbles were used. We concluded that LSI has potential to detect dysfunctional microvessels amongst the flowing microvascular network in vivo when using microbubbles as exogenous contrast agents in LSI method.

10059-65, Session PMon

UV fluorescence excitation spectroscopy as a non-invasive predictor of epidermal proliferation and clinical performance of cosmetic formulations

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The epidermis is the outermost layer of skin and is composed of cells primarily containing keratin. It consists of about ten layers of living cells (keratinocytes) and ten layers of dead cells (corneocytes). These cells

are continually shed from the outside and replaced from the inside in a process called desquamation which is controlled by two biological events – proliferation and differentiation.

One method to non-invasively study biological changes in the skin is by using fluorescence excitation spectroscopy. Signals that can be observed include the fluorescence from tryptophan, pepsin-digestible collagen cross-links, collagenase digestible collagen cross-links, and elastin cross-links. The magnitude of the tryptophan fluorescence signal has been linked to changes in skin proliferation, cell turnover, changes in epidermal thickening, and ultimately with overall signs of skin aging. We hypothesize that increases in this fluorescent signal could be used to assess the potential activity of cosmetic anti-aging compounds to deliver a benefit to skin.

Retinol and glycolic acid are two commonly used cosmetic active agents used to deliver a cosmetic skin benefit by inducing epidermal proliferation and exfoliation, respectively. Previous work has demonstrated that application of formulations containing these compounds to skin for about 2-4 weeks results in an increase in tryptophan fluorescence. In this study we present the results of a double-blind placebo controlled study that aims to correlate changes in tryptophan fluorescence with biological (epidermal thickening and Ki67 expression) and clinical (dermatologist grading) performance. Our data shows that tryptophan fluorescence could be used as a non-invasive early indicator of skin anti-aging benefits.

10059-66, Session PMon

Dual modality breast imaging system: combination digital breast tomosynthesis and diffuse optical tomography

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We have developed a digital breast tomosynthesis(DBT) system in Korea Electrotechnology Research Institute (KERI) and installed and been under clinical test in Asan Medical Center (AMC) [1]. Recently we develop a frequency domain diffuse optical tomography(DOT) system to combine with the DBT system for diagnostic accuracy improvement by using joint breast cancer diagnosis. It is well known that a DBT/DOT combination system was first demonstrate at the Massachusetts General Hospital(MGH) and recently V. Krishnaswamy et al proposed a low-cost continuous wave NIRST-DBT system using large area silicon photodiode at Dartmouth College [2]. In this work, we adapted frequency domain DOT system with the three laser diode modules with 785 nm, 808 nm and 850 nm. Two optical MEMS switches are used to deliver light to 64 specific positions in a source paddle. The three one-tone modulation light sources are detected simultaneously by 40 avalanche photodiodes installed in a detection paddle after passing an optical phantom. We use an In-phase(I) and quadrature(Q) demodulator to obtain amplitude and phase of the signal. The 40 IQ signals are obtained simultaneously using NI PXIe 6368 boards. We also develop a image reconstruction program using finite element method. Figure 1 shows a DBT and DOT images of phantom with cylinder hole with 20-mm diameter, which is filled by 15-mm thick acetal cylinder with respect to vertical distance from top surface.

[1] H-S. Park, Y-S. Kim, H-J. Kim, Y-W. Choi and J-G. Choi, 'Optimization of configuration parameters in a newly developed digital breast tomosynthesis system, J Radidation Research, 55, 589-599, 2013

[2]V. Krishnaswamy et al, "A digital x-ray tomosynthesis coupled near infrared spectral tomography system for dual-modality breast imaging," Opt. Express, 20, 19125-19136, 2012

10059-67, Session PMon

Time-domain Hemoglobin Diffuse Optical Tomography of Breast: a pilot study

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Abstract: The objective of this study was to primary certify the validity of a time-domain diffuse optical tomography system for breast tumor diagnosis. A time-domain diffuse optical tomography system is proposed based on the multi-channel time-correlated single-photon counting technique?Aligning 32 coaxial fibers around the tissue surface equally; the system scans objects in a parallel-beam mode analogous to X-ray CT so that the time-resolved projections at different incident positions can be obtained?By applying the relevant iteration reconstruction algorithm?promising images have been produced from measurements on one patient?Reconstructed images correctly discloses the mass location and various of contrast mechanisms (oxy-hemoglobin, deoxy-hemoglobin, total hemoglobin and blood oxygen saturation), but the reconstructed images discloses the poor spatial resolution and mass size larger than the ultrasound and magnatic resonance imaging.

Conclusions: The results primary indicate this system works reliably, and we need to measure more cases to assess the feasibility of using this system to distinguish between benign and malignant cases?

10059-68, Session PMon

Wide-field fluorescence diffuse optical tomography with epi-illumination of sinusoidal pattern

Feng Gao, Tongxin Li, Weiting Chen, Caixia Qi, Panpan Yan, Huijuan Zhao, Tianjin Univ. (China)

We present a wide-field fluorescence tomography with epi-illumination of sinusoidal pattern. A DMD projector is employed as a spatial light modulator to generate independently wide-field sinusoidal illumination patterns at varying spatial frequencies on a sample, and the emitted photons at the sample surface were captured with a EM-CCD camera. This method results in a significantly reduced number of the optical field measurements as compared to the point-source-scanning ones as well as preservation of the high frequency components as similarly demonstrated in structure-illumination microscopy, and thereby achieves a fast data acquisition and a potentially improved spatial resolution. Phantom experiments for a combination of the multiply frequencies validate the ability of the method to enhance the reconstruction.

10059-69, Session PMon

Blood flow measurement of human skeletal muscle during various exercise intensity using diffuse correlation spectroscopy (DCS)

Yuya Murakami, Yumie Ono, Masashi Ichinose, Meiji Univ. (Japan)

Quantitative measurement of muscle blood flow during actual exercise can offer useful information for athletes and researchers in the field of sport medicine, because blood supply to active skeletal muscles is one of the important determinants of exercise performance. We here investigated the

potential of diffuse correlation spectroscopy (DCS), an emerging optical modality that is suitable for noninvasive quantification of microcirculation level in deep tissue, to assess the blood flow dynamics of active skeletal muscle. Seven healthy subjects conducted continuous hand grip exercise at 0.5 Hz for 3 minutes with varied intensities of 10, 20, 30, and 50 % of maximal voluntary contraction (MVC). DCS could detect the time-dependent increase of the blood flow response of the forearm muscle for continuous exercises, and the increase ratio of the mean blood flow through the exercise period showed good correlation with the exercise intensities. We also compared blood flow responses detected from DCS with two different photon sampling rates and found that an appropriate photon sampling rates should be selected to follow the wide-ranged increase in the muscle blood flow with continuous exercise. Our results suggest the possibility for utilizing DCS in a field of sports medicine to noninvasively evaluate the dynamics of blood flow in the active muscles.

10059-71, Session PMon

An endoscopic diffuse optical tomographic method with high resolution based on the improved FOCUSS method

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Endoscopic DOT has the potential to apply to cancer-related imaging in the stomach, cervix, prostate, bladder, etc. While the DOT has relatively large tissue penetration depth, the endoscopic DOT is limited by the narrow space of the internal tubular tissue, so as to the relatively short source-detection distance and small penetration depth. Because some adenocarcinomas including cervical adenocarcinoma are located in deep canal, it is necessary to improve the imaging resolution under the limited measurement condition. To improve the spatial resolution, in this paper, we developed a new FOCUSS algorithm along with the image reconstruction algorithm with the effective detection range (EDR). This algorithm introduces the re-weighted minimum norm. The standardization is involved in the recursive process to enhance the localization ability. The shrinking method is cut down the computation burden of image reconstruction. For a typical inner size and optical properties of the cervix-like tubular tissue, comparing to the traditional image reconstruction algorithm based on the EDR, the adoption of the new FOCUSS method results in high resolution. Images reconstructed from the simulation data demonstrate that the proposed method achieves equivalent image quality to that obtained from the traditional method based on EDR when the targets near the boundary, and with higher spatial resolution and fidelity when the targets far from the boundary .The fidelity of reconstructed absorption and reduced scattering coefficient can be up to 90% and 80%, respectively. Furthermore, the reconstruction results show that multi-targets with different depths can be reconstructed correctly. The proposed method will be useful to the development of endoscopic DOT technologies for cancer detection in tubular organs.

10059-72, Session PMon

Assessment of using ultrasound images as prior for diffuse optical tomography regularization matrix

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Imaging of tissue with Ultrasound-guided diffuse optical tomography (DOT) is a rising imaging technique to map hemoglobin concentrations within tissue for breast cancer detection and diagnosis. The accurate recovery of images requires an effective image reconstruction method. We illustrate a method in which ultrasound images are encoded as prior for regularization of the inversion matrix. The framework of this work is based on image reconstruction package “NIRFAST”. We compare the results of this method to the dual-zone mesh conjugate gradient reconstruction method based on born approximation, which was developed in our laboratory. Results were evaluated using phantom experiments and patients’ data. This method improves differentiation between malignant and benign cases by increasing malignant to benign absorption ratio. The results show also improvements in lesion shape as well as the spatial resolution of the DOT reconstructed images.

10059-73, Session PMon

Miniaturized spectrometers using integrated optics

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Two new spectrometer ideas based on integrated optics will be presented. The first one is called interlaved arrayed waveguide grating (AWG) spectrometer provides large bandwith and high resolution for a very compact size. The second spectrometer is called ultra-high resolution AWG provides 1 pm (around 100 MHz) of resolution for only 2x2.5 cm device size at 1.3 μm. For the interlaved AWG spectrometer the primary AWG has narrow closely spaced passbands (that equal the final desired channel spacing) that repeat N times in the desired wavelength range, using the frequency-cyclic nature of the AWG. The channel spacing of the secondary AWGs should be equal to the free spectral range (FSR) of the primary AWG. In this configuration the FSR of the secondary AWGs defines the FSR of the overall configuration whereas the channel spacing (resolution) of the primary AWG defines the overall system resolution. The ultra-high resolution AWG spectrometer is formed of several directional couplers and circular and straight waveguides. The input light will be tapped out by a certain amount at specific locations. The path length difference between first and the last tapped light could be around 30-40 cm which corresponds to a wavelength resolution of 1 pm (100 MHz). The tapped light will be combined in a star coupler region for constructive interference and later focusing. For an FSR of 4 GHz at 1.3 μm the estimated device size will be around 2x2.5 cm for 500 micron of bending radius of the circular waveguides.

10059-74, Session PMon

Observation of chest tumor using diffuse optical spectroscopy: time-varying Indocyanine green concentration in rabbit model

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This experiment was conducted by using the diffuse optical spectroscopy based on near-infrared light. The near-infrared light in the water window was used to see the change of molecular concentration in the living tissue. The experiment subject was New Zealand rabbits weighing 3 ± 0.3 kg. VX2 tumor cells were injected into the inside of the chest wall of rabbits. The concentration of indocyanine green (ICG) has been observed once every three days, after the size of the pleural tumor grew up over 1cm. We used five different wavelengths (732, 758, 805, 840, and 880 nm) with known ICG spectrum. The distance between light source and detector probes was

fixed by 1 cm. The probes were placed on the skin right above the tumor with an aid of laparoscope. ICG was injected into rabbits via ear vein. The diffused light was measured through the tumor with time course using a spectrometer. These measured data enabled us to observe the change of ICG concentration in real time with respect to the baseline without ICG. ICG was present longer in tumor compared to normal tissue. This phenomenon is thought to be due to the excessive angiogenesis in the tumor tissue. Since this method can be applied to other cases easily, it is thought that there is a possibility of cancer screening with less cost and simple equipment.

10059-75, Session PMon

Brain perfusion assessment based on analysis of a derivative of optical signals measured after administration of an optical contrast agent at large source-detector separation

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We applied custom made high density diffuse optical tomography system in measurements of inflow and washout of an optical contrast agent into a brain. Thus, a brain perfusion parameters can be estimated. The indocyanine green (ICG) was injected into a vein of a healthy volunteer, while the optodes were positioned on a head above a visual cortex. The optodes, in a form of a grid, made a combination of 16 sources and 12 detectors separated by distances from 1.5 cm to 8 cm. We observed appearance of the ICG as a drop in optical signals at 735 nm, caused by increased absorption. In healthy subject the ICG inflows into the brain and is relatively quickly washed out from the brain. Moreover, it appears slightly faster in the brain than in extracerebral tissues. Thus the optical signal carries information about the inflow of the ICG into the brain and into extracerebral tissues as well.

We analyzed the derivative of attenuation of the optical signals in respect to source-detector separations. It can be noted, that the amplitude of the derivative, so the speed of the inflow of the ICG, increases with increasing source-detector separation. We presume, that this effect is related to increased contribution of information from the brain in signals measured at large source-detector separations. Thus, the speed of the inflow of the ICG increases with increasing distance between source and detector. It suggests, that increase of the source-detector separation improves the sensitivity of the system to a brain perfusion changes.

10059-76, Session PMon

Design and fabrication of a multi-layered solid dynamic phantom: Validation platform on methods for reducing scalp-hemodynamic effect from fNIRS signal

Hiroshi Kawaguchi, Yukari Tanikawa, Toru Yamada, AIST (Japan)

The scalp-hemodynamics contaminates the signal of functional near infrared spectroscopy. Numerous methods have been proposed to reduce the contamination, however, a golden standard has not been found yet. Here, we constructed a multi-layered solid dynamic phantom to validate such methods experimentally.

The phantom consists of 4 layers corresponding to epidermides, dermis and skull (upper dynamic layer), cerebrospinal fluid and brain (lower dynamic layer) and the thickness of each layer was 0.3, 10, 1 and 50 mm, respectively. The epidermides and cerebrospinal fluid layers were made of a polystyrene and acrylic board, respectively. Both the dynamic layers were made of the epoxy resin. An infrared dye and titanium dioxide were mixed to match their absorption and reduced scattering coefficients (μa and μs') with biological values, respectively. Both base parts of upper and lower dynamic layer has a slot for a laterally sliding bar that holds an absorber piece. The bar was laterally moved using a programmable stepping motor.

The optical properties of dynamic layers were estimated from the transmittance and reflectance by the Monte Carlo lookup table method. The estimated coefficients (μa, μs') for lower and upper dynamic layers, (0.015, 2.207) and (0.008, 1.919) mm-1 at a wavelength of 800 nm, respectively, approximately coincided with biological tissue values. The preliminary NIRS measurement using the fabricated phantom showed the decrease in detected intensity when the absorber was positioned below the area between source and detector probes in any cases that the absorbers in the upper and/or lower dynamic layer were moved.

10059-77, Session PMon

Identification and quantitative evaluation of pathological tissue fibrosis using Mueller matrix microscope

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Fibrosis happens during various pathological processes, such as liver cirrhosis and cancer, pulmonary fibrosis and so on. Currently, histopathological examination is regarded as the golden diagnosis criterion, but different doctors with discrepancy in knowledge and experience may obtain different qualitative conclusions. Up to a point, quantitative evaluation of the pathological tissue fibrosis can be of great service to diagnosis and precision medicine of diseases. Mueller matrix polarimetry is capable of probing comprehensive microstructural information of tissue samples and is a valuable attempt in fibrosis quantification. In this paper, we apply the modulus design Mueller matrix microscope set up in our previous study to the pathological tissue slices of different diseases and adopt the Mueller matrix polar decomposition (MMPD) and Mueller matrix transformation (MMT) parameters for quantitative analysis. In addition, we use the Monte Carlo simulation to analyze how the fibrosis changes in pathological processes affect the microscopic Mueller matrix parameters. The experimental and Monte Carlo simulated results show good consistency. Fibrosis in different tissues and stages have different characteristic features of Mueller matrix elements. Different Mueller matrix parameters are used for quantitative evaluations of fibrous structures including parameters of retardation, fast axis angle and so on. The results presented in this paper indicate that the Mueller matrix microscope and the orientation independent Mueller matrix parameters can provide additional information for the identification and quantitative evaluation of the pathological fibrosis tissues. Thus, it has a good application prospect in auxiliary diagnoses.

10059-20, Session 5

Individual response to neoadjuvant chemotherapy assessed with optical mammography in patients with breast cancer *(Invited Paper)*

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We report an optical mammography study on 10 patients with breast cancer who underwent neoadjuvant chemotherapy. The optical mammography instrument operates in a transmission geometry, with the illumination and

collection optical fibers scanned in tandem over the slightly compressed breast. Continuous-wave illumination and spectrally resolved detection cover the spectral band 650-850 nm. Optical mammograms were collected on both breasts before chemotherapy treatment and at every chemotherapy infusion for a total number of 7-18 optical mammography sessions over a period of 17-30 weeks, depending on the individual patient. Pathologic response was assessed after surgery and each patient was classified as a complete (pCR: no remaining cancer), high level (PR1: cancer reduction by more than 50%), or low level (PR2: cancer reduction by less than 50%) responder. A cumulative response index (CRI) was computed at each optical mammography session on the basis of the progression of total hemoglobin concentration ([HbT]) or hemoglobin saturation ([SO2]) at the tumor region of interest (ROI). We found that patients who respond to neoadjuvant chemotherapy show a stronger decrease in the [HbT] and [SO2] at the tumor ROI with respect to non-responders. Such decreases were captured by the optical mammography CRI, which showed the ability to successfully discriminate responders (pCR and PR1) from non-responders (PR2) at a 40% therapy time point and potentially earlier. We further discuss the role of systemic effects of neoadjuvant chemotherapy, which were investigated by monitoring the hemoglobin concentrations in blood and the optical mammograms of the contralateral, healthy breast.

10059-21, Session 5

Development of time-resolved reflectance diffuse optical tomography for breast cancer monitoring

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We report a time-resolved reflectance diffuse optical tomography (RDOT) which has been developed to measure the responses during chemotherapy for breast cancer at bedside. This system irradiates the breast with a three-wavelength pulsed laser (760, 800 and 830nm) through the source fiber specified by an optical switch. The light collected with detector fibers is guided to a detector unit consisting of variable attenuators and photomultiplier tubes. The 13 irradiation and 12 detection points are set to the measurement area of 50mm by 50mm of the handheld probe. The data acquisition time to obtain the temporal profiles within the measurement area is about 2 minutes. Since the RDOT has sufficient dynamic range to acquire the temporal profile within the entire measurement area, topographic and tomographic images of the optical properties are obtained by using appropriate data sets. The topographic images are obtained from the optical properties determined for each source-detector pair by using curve fitting method based on the photon diffusion theory. The tomographic images are reconstructed with an iterative image reconstruction method. Since the topographic images are obtained in a few seconds after the measurement is finished, these images can be checked immediately. In phantom experiments, a cylindrical target (15mm in diameter and 15mm in height) embedded in the background medium was successfully reconstructed. In preliminary clinical measurement, the tumor of a breast cancer was imaged as high hemoglobin concentration region. The results demonstrated the potential that the RDOT can be used to evaluate the effectiveness of breast cancer chemotherapy.

10059-22, Session 5

Change in tumor hemoglobin concentration during neoadjuvant chemotherapy may predict pathological response in ER-negative breast cancer, but not in ER-positive breast cancer

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PURPOSE

Response monitoring with diffuse optical spectroscopic imaging (DOSI) during neoadjuvant chemotherapy (NAC) in breast cancer is promising, but knowledge of breast cancer subtype is essential. The aim of the present study was to evaluate the relevance of breast cancer subtypes for monitoring of therapy response during NAC with DOSI.

METHODS:

Evaluation included 83 women with operable breast cancer. DOSI were performed before and after 2 course of NAC. Tumor total hemoglobin concentration (tHb) was quantified using breast imaging system with TRS10/20 (Hamamatsu K.K., Japan). Tumor response at surgery was assessed dichotomously pCR (ypTis/0) and non-pCR. Receiver operating characteristic (ROC) analyses were employed to determine associations with pathological response.

RESULTS:

A pCR was seen in 15 of 83 tumors. (8 of 48 ER-positive and 7 of 35 ER-negative tumors). The area under the ROC curve was 0.50 (0.35-0.65) for ER-positive, 0.82 (0.66-0.93) for ER-negative tumors. We found no association between age, stage, histology, or baseline tHb.

CONCLUSION:

Response monitoring with DOSI during NAC in breast cancer seems feasible, but is dependent on the breast cancer subtype. DOSI may predict response in ER-negative tumors, but seems less accurate in ER-positive tumors.

10059-23, Session 5

Breast Cancer Detection using Ktrans MRI imaging guided near infrared spectroscopy tomography

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This is the first human pilot examination of unenhanced–MRI to guide NIRST for breast cancer imaging. Magnetic resonance (MR) guided Near Infrared Spectral Tomography (NIRST) has the potential to provide high-resolution images for breast cancer diagnosis. However, most of the previous studies are based on MRI T1-weighted dynamitic contrast enhanced (DCE) images that need to inject contrast agent with a concern of the risk of immediate adverse reactions and contraindications in patients with impaired renal function. In this study, the non-contrast T2-weighted MR images were

directly incorporated into the inversion matrix regularization to reconstruct optical images without segmentation. A total of 25 patients were involved in this study, with 17 pathologically confirmed malignancy and 8 benign. All patients were imaged by a MRI guided NIRST system which can acquire MR and NIRST image data simultaneously. The NIRST system includes six frequency domain FD wavelengths (spanning from 660nm to 850nm) and three continuous wave (CW) wavelengths (900nm to 950nm). The NIRST reconstruction was performed based on open source platform, NIRFAST. The statistical results revealed that the estimated HbT values based on T2-weighted MR-guided optical image reconstruction can differentiate the malignant from benign groups with P < 0.001.The mean contrast values were 1.77 ± 0.87 and 0.86 ± 0.22 of the malignant and benign cases, respectively. The overall accuracy of the imaging diagnosis using ROC analysis improved from 79% (T2-Weighted MRI only) to 92% with NIRST combined with T2-Weighted MRI.

10059-24, Session 5

Complex evaluation of metabolism and blood supply breast cancer in the preoperative treatment

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Modern methods of breast cancer treatment suggest the evaluation of not only structural but also metabolic tumor changes in the course of treatment.

The data of oxygenation dynamics and the state of breast tumor vascular channel were selected as the criteria for metabolic response. The aim of the study was complex assessment of metabolic dynamics and breast tumor blood supply in the period of neoadjuvant polychemotherapy (NAPCT). 27 patients with breast cancer were treated with NAPCT. Before NAPCT and after the first course the oxygenation level and tumor blood supply was obtained.

Tumor oxygenation level was determined employing optical diffuse spectroscopy (DOS) setup IAP RAS (Nizhny Novgorod, Russia). Tumor blood supply was performed by ultrasonography employing “Medison Accuvix V20” setup in power Doppler mode. 18 patients completed the whole course of treatment. The DOS was used for 8 patients, the ultrasonography for 5 patients and both methods were employed for other 5. 18 patients were operated for mastectomy. Tumor response was determined in accordance with Miller I.D., Payne S. classification. The DOS study of oxygenation level dynamics and the ultrasonography tumor blood supply study revealed variable changes depending on the state of tumor response. In patients with the absence or weak therapeutic pathomorphosis oxygenation level in tumors after the first course of NAPCT decreased, but the number of vessels in tumors didn’t essencially change. In case of significant therapeutic pathomorphosis after the NAPCT oxygenation level in tumors increased and the number of vessels in tumors considerably decreased.

Tumor response to chemotherapy is correlated with the dynamics of tumor oxygenation and the dynamics of tumor blood supply in course of treatment which can be employed to predict the effects of therapeutic procedures.

10059-25, Session 5

Neural-network based classification for predicting pathological complete response to neoadjuvant chemotherapy in breast cancer patients with dynamic diffuse optical tomography

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Neoadjuvant chemotherapy (NACT) has become a well-stablished therapy in the treatment of patients with locally advanced or primarily inoperable breast cancer. In recent years several groups, including our team, have shown evidence that diffuse optical tomography (DOT) may play an important role in triaging patients that respond to therapy from patients that do not respond from therapy. For example, analyzing the data of 34 patients we have reported on sensitivities and specificities larger than 0.85 for predicting treatment outcome. For that study we had employed a continuous-wave DOT system that imaged the hemodynamic response to a breath hold in both breasts simultaneously just before treatment initiation and 2 weeks later. Changes in the dynamic imaging features were correlated with 6-month treatment outcome

In the study at hand, we implemented a neural-network–based classification scheme to further improve on the accuracy for predicting responders. In particular, we employed a two-layer feed-forward neural network with 4 inputs (changes in oxy-hemoglobin concentration, total hemoglobin concentration, water fraction, and ER status), 10 hidden neurons and 1 output to predict complete pathological responses. Using data from now 42 patients who underwent to the same NACT treatment, we were able to achieve sensitivities and specificities of >0.95. We are actively enrolling patients and will report on our most up-to-date findings.

10059-26, Session 6

A portable, 12-wavelength parallel near-infrared spectral tomography (NIRST) system for efficient characterization of breast cancer during neoadjuvant chemotherapy

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Neoadjuvant chemotherapy (NAC, i.e. applied prior to surgery) is prescribed for breast cancers when patients have tumor size larger than 3 cm and/or multiple lesions. In order to assess responses to this treatment, a portable system for near infrared spectral tomography (NIRST) was developed to monitor women receiving this therapy. The system uses both frequency domain and continuous wave light source technologies, for simultaneous acquisition of 12 wavelengths, covering the wavelength range of 661–1064nm. The system design significantly speeds up acquisition, as compared with previous sequential acquisition schemes, and yet achieves a wider bandwidth of wavelengths. An adjustable interface was designed to fit various breast sizes and shapes. Images of oxy- and deoxy-hemoglobin, water, lipid, and scattering components were reconstructed using a 2D FEM approach. A group of normal subjects and cancer patients with breast abnormalities were imaged. Higher total hemoglobin and water contents

were estimated in radiographically dense breasts versus those with less dense breast, from the optical images of normal subjects. The imaging system was able to detect breast tumors with a size of 1.5 cm and larger, with a tumor to surrounding normal tissue contrast ratio of 1.4 in total hemoglobin and 1.2 in water, respectively. An imaging study involving a larger cohort of breast NAC patients is ongoing.

10059-27, Session 6

Development of multi-parametric prediction model for chemotherapy efficacy based on diffuse optical and correlation spectroscopy on breast cancer xenografts

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Personalized breast cancer treatment is currently difficult due to the lack of reliable methods to predict the effectiveness of treatments. Several clinical studies have demonstrated that diffuse optical methods have potential to predict neoadjuvant chemotherapeutic efficacy in breast cancer. However, most studies did not have enough number of subjects to take advantage of full potential of diffuse optics: oxygen metabolism and multi-parametric analysis. A preclinical study can be an alternative to identify important parameters for developing a multi-parametric prediction therapeutic model since it provides measurements of sufficient number of subjects at a faster pace than clinical trials . Furthermore, the study can provide a platform to validate diffuse optical methods using invasive gold standard measurements. In this vein, we have simultaneously measured blood flow, total hemoglobin concentration, blood oxygen saturation, and tissue scattering of 125 murine breast cancer xenografts with a diffuse correlation spectroscopy and a diffuse optical spectroscopy. Six different treatment regimens involving doxorubicin, cyclophosphamide, and paclitaxel at clinically relevant dose were investigated against control group with saline injection. In terms of blood flow, strong correlation between early blood flow changes and treatment outcome was observed for effective treatments. In addition, individual blood flow changes at day 3 or 7 after drug administration demonstrated relatively high differentiation between tumors with effective treatment and no treatment. We hypothesize that the combination of multiple parameters will significantly enhance the capacity to predict early, and will develop a multi-parametric therapeutic prediction model for breast cancer for early response detection.

10059-28, Session 6

Spatial frequency domain imaging for monitoring palpable breast lesions

Constance M. Robbins, James F. Antaki, Jana M. Kainerstorfer, Carnegie Mellon Univ. (United States)

Clinical and self-examination of the breast aim to detect malignancies by touch, based on the relative stiffness of the lesion to the surrounding healthy tissue. However, these methods lack specificity and are associated with unnecessary biopsies. In addition to stiffness, breast cancer lesions can be differentiated by their relative optical properties – due to the greater vascularization, hence hemoglobin concentration. This project aims to develop a point-of-care technique to identify, measure, and track palpable breast lesions using a simple light source (projector) and camera. For this, we use a spatial frequency domain imaging (SFDI) approach. This technique is a wide-field optical imaging method that uses diffusely reflected illumination patterns (multiple spatial frequencies) and can map absorption and scattering properties of superficial layers of tissue. Using near-infrared light, maps of hemoglobin concentration can be created. We performed pilot studies on rigid silicone phantoms with embedded inclusions with higher absorption coefficient than surrounding tissue. Inclusions were discernible at depths up to 6 mm. We will further present studies using compressible phantoms that mimic the mechanical and optical properties of breast tissue having stiffer, higher absorbing inclusions, which mimic breast cancer lesions. With this, we will demonstrate that mechanical compression of the phantom with an optically clear lens in the region of interest will bring deeper lesions within the depth of field of the imaging system, making SFDI a viable tool for monitoring vascularization of palpable breast lesions.

10059-78, Session 6

Differential diagnosis of breast masses in south Korean premenopausal women using diffuse optical spectroscopic imaging (DOSI)

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No Abstract Available

10059-29, Session 7

Bayesian design of structured illumination for diffuse optical tomography of mouse brain

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For functional neuroimaging, existing small-animal diffuse optical tomography (DOT) systems either do not provide adequate temporal sampling rates or have sparse spatial sampling. To achieve adequate frame rates (1-10 Hz) we have constructed a system using sCMOS detection-based DOT, with asymmetric measurements, with many (>10,000) detectors and fewer (<100) structured illumination patterns (using a digital micromirror device: DMD). Flexibility of the DMD requires a rational, scalable method to identify optimal pattern sequences. For instance, will a sparse set of points, single stripes, or square waves provide the best combination of resolution, noise, and depth sensitivity? To address this question, we needed to develop a way to predict the performance of a pattern sequence. Herein we employed a Bayesian approach to estimate the uncertainty in reconstructed images given a candidate illumination scheme. Following a Bayesian view, the posterior probability distribution given the pattern sequence and (random) measurements encodes the reconstructed image (the posterior's mode) and its uncertainty at each voxel. Since every pattern sequence produces an associated posterior uncertainty map, one way of ranking each candidate sequence's performance is by its posterior variance averaged across the region(s) of interest. Using this approach, we found that square wave sequences outperformed single-stripe sequences, which in turn outperformed single-point sequences. We are also establishing alternate ranking strategies that consider depth sensitivity, resolution, and absolute image error. In summary, Bayesian computation of posterior distributions is

a powerful approach to quantifying imaging performance and appears to have great utility in optimizing DOT measurement sets.

10059-30, Session 7

Radiance Monte-Carlo for image reconstruction in diffuse optical tomography based upon the transport equation

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Diffuse Optical Tomography (DOT) is a technique which seeks to image spatially varying optical properties of biological tissues from measurements of light transmission through the medium. Recovery of the parameters of interest is typically achieved by the solution of a model-based inverse problem, whereby an objective function representing the difference between the measured data and the a suitable forward model is minimised.

Owing to its modest computational requirements, the diffusion approximation (DA) to the radiative transport equation (RTE) is the usual choice of forward model employed in the inverse problem. However, the DA is invalid in many contexts of increasing interest, including in the case of short source-detector separations of increasing use in high-density DOT instruments, and in non-scattering regions such as the CSF layer surrounding the brain. Recent advances in parallel computing have made Monte-Carlo solutions to the RTE viable as a forward model, but until now it has not been shown how MC solutions to the RTE can be properly and efficiently used to solve the inverse problem.

In this work we introduce a new Monte-Carlo technique to estimate the radiance distribution in a medium according to the RTE. We demonstrate how to efficiently form gradients of the forward model, and thus how to employ this technique as part of the inverse problem. We are able, for the first time, to demonstrate computationally viable full three-dimensional reconstructions in DOT based upon the transport equation, overcoming the limitations of the diffusion approximation.

10059-31, Session 7

Statistics of photon penetration depth in diffusive media

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The study of photon migration through highly scattering media opens the way to the non-invasive investigation of biological tissues well below the skin surface. When the medium is addressed in reflectance geometry, light is injected and collected from the same side of the surface. Then, a key issue is to increase as much as possible the depth reached by migrating photons. Depth information is crucial, for instance, in brain functional imaging or in neuro-monitoring, where a key challenge is the extraction of specific brain-cortex signals out of the overwhelming systemic superficial contamination (e.g. scalp, skull and cerebrospinal fluid).

In this paper we calculated, for a laterally-infinite slab, the time-resolved (TR) probability density functions (PDFs) for the maximum depth reached by detected photons. From PDFs it is possible to calculate the mean value of the maximum penetration depth and, then, to have an estimation of the mean value at which detected photons have undergone scattering events. We did the same calculations for the continuous-wave (CW) domain. In contrast to the TR approach, in the CW domain PDFs and the mean maximum penetration depth depend on the absorption coefficient of the diffusive medium. In order to perform these calculations, we exploited both a Monte Carlo code and the diffusion approximation of the Radiative Transport Equation. Work is in progress to evaluate the same quantities in the frequency domain and in a diffusive heterogeneous medium.

10059-32, Session 7

Computationally-efficient and analytical radiative transport Jacobian models for transport-regime fluorescence mesoscopic tomography

Golam Kibria Chowdhury, Univ. of Alberta (Canada); André Liemert, Institut für Lasertechnologien in der Medizin und Messtechnik (Germany); Roger J. Zemp, Univ. of Alberta (Canada)

Optical fluence calculations using the diffusion approximation are poor within the transport regime and near boundaries. This approximation cannot be used to reconstruct high-resolution images adequately in the transport regime (depths of a few transport mean-free pathlengths). Monte Carlo simulations are inherently stochastic and computationally expensive. We make use of a recently published exact and efficient eigenvalue formalism of the radiative transport equation (RTE) in a semi-infinite medium using higher order PN approximation to compute the Jacobian matrix for fluorescence tomography. Solutions for subsurface fluence from a pencil or Gaussian beam are coupled with diffuse reflectance models for an isotropic point-source in a semi-infinite medium to compute the Jacobian matrix and forward model. RTE models are compared with Monte Carlo simulations. Monte Carlo simulations are used to create software phantoms for reconstruction. Transport-regime inverse problems are illustrated with significant advantages over Diffuse Optical Tomography, especially when subsurface fluorophores are close to the transport mean-free path, where the diffusion approximation encounters a singularity which is avoided with the RTE models.

10059-33, Session 7

Real-time single spatial frequency domain imaging by snapshot multiple frequency demodulation technique

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We described and validated the Single Snapshot Multiple Frequency Demodulation method applied to the Spatial Frequency Domain Imaging. In one hand, SSMD can extract the DC and multiple AC components of different orientation, modulating frequencies and amplitudes from a single snapshot at once. SSMD hence expands the range over which MTF can be correctly measured than that with the current demodulation approaches. This is critical for real time imaging of dynamic turbid media and avoids motion artefacts. In another hand, comparing to the conventional three-phase, Hilbert demodulation methods or the SSOP method, SSMD achieves the best performance at a moderate to high noise levels in terms of noise suppression. SSMD effectively removes the oscillations in the reconstructed optical property maps when the three-phase demodulation is employed. In addition, as the probing depth of SFDI varies with the modulation frequency, the plurality of the modulation frequencies within a single snapshot in SSMD enables the characterization of the optical properties of the turbid medium at different depths simultaneously at once as well.

10059-34, Session 8

A novel approach for the time-domain fluorescence imaging of a semi-infinite turbid medium

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The feasibility of the reconstruction of a fluorophore embedded in a semi-infinite media was evaluated by a proposal novel strategy with a few boundary time-resolved experimental data. In this strategy, we neglect the presence of the fluorophore for the excitation light, therefore, we used the Green function for a homogeneous semi-infinite media. The propagation of near-infrared light in tissue is modelled by the time-dependent radiative transfer and the diffusion equations. The potential of implementing the level set with contrast value and the Sparsity regularization techniques was investigated in order to estimate more accurately the shape and position of the fluorophore and the lifetime coefficients. In addition, a heuristic optimal geometry configuration of detectors/sources positions was tested.

Assuming that the concentration of the fluorophore is equal to zero in the excitation light equation, it is possible to use the Green function associated with the homogeneous semi-infinite media. When the propagation of light was modelled with the radiative transfer equation (RTE) or the diffusion equation (DE), we have used the heuristic Green function with an Extrapolated-Boundary condition (EBC), and the Green function with an EBC, respectively, both of them reported in the literature. The emission fluence was calculated numerically using the RTE or the DE accordingly. We point out that we have not used Perturbation theory such as Born or Rytov approximations previously published. The inverse imaging problem was solved using the Landweber-Kaczmarz and a quasi-Newton techniques with adjoint fields. The sparsity reconstruction was retrieved with a fast and novel algorithm.

10059-35, Session 8

A three-step reconstruction method for fluorescence molecular tomography based on compressive sensing

Yansong Zhu, Abhinav K. Jha, Johns Hopkins Univ. (United States); Jakob K. Dreyer, Univ. of Copenhagen (Denmark); Hanh N. D. Le, Jin U. Kang, Johns Hopkins Univ. (United States); Per E. Roland, Univ. of Copenhagen (Denmark); Dean F. Wong, Arman Rahmim, Johns Hopkins Univ. (United States)

Fluorescence molecular tomography (FMT) is a promising tool for real time in vivo quantification of neurotransmission (NT) as we pursue in our BRAIN initiative effort. However, the acquired image data are noisy and the reconstruction problem is ill-posed. Further, while spatial sparsity of the NT effects could be exploited, traditional compressive-sensing methods cannot be directly applied as the system matrix in FMT is highly coherent. To overcome these issues, we propose and assess a three-step reconstruction method. First, truncated singular value decomposition is applied on the data to enforce incoherence. The resultant image data are input to a homotopy-based reconstruction strategy that exploits sparsity via L1 regularization. The reconstructed image is then input to a maximum-likelihood expectation maximization (MLEM) algorithm that retains the sparseness of the input estimate and improves upon the quantitation by accurate Poisson noise modeling. The proposed reconstruction method was evaluated in a three-dimensional simulated setup with fluorescent sources in a cuboidal scattering medium with optical properties simulating human brain cortex (reduced scattering coefficient: 9.2 cm^-1, absorption coefficient: 0.1 cm^-1) and tomographic measurements made using pixelated detectors. In different experiments, fluorescent sources of varying size and intensity were simulated. The proposed reconstruction method provided accurate estimates of the fluorescent source intensity, with a 60% lower mean square error in average compared to the pure-homotopy method for all considered source intensities and sizes. Further, compared with MLEM-only algorithm, the proposed method reconstructed substantially more accurate fluorescence distribution. The proposed method shows considerable promise and will be tested using more realistic simulations and experimental setups.

10059-36, Session 8

Gaussian kernel based anatomically-aided diffuse optical tomography reconstruction algorithm

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Image reconstruction in diffuse optical tomography (DOT) is challenging because its inverse problem is a non-linear, ill-posed and ill-conditioned. Anatomical guidance from high spatial resolution imaging modalities can substantially improve the quality of the reconstructed DOT images. In this paper, inspired by the kernel methods in machine learning, a new approach to introducing anatomical information into the DOT image reconstruction algorithm is described. In this method, optical absorption coefficient at each finite element node is represented as a function of a set of features obtained from anatomical images such as computed tomography (CT). The kernel based image model is directly incorporated into the forward model of DOT, which exploits the sparseness of the image in the feature space. Comparing to other approaches to include structural priors in regularization matrices, such as soft prior and hard prior methods, the proposed method does not require user image segmentation of distinct regions and regularization matrix. The algorithm has been validated with numerical simulations of 3D DOT reconstruction using synthetic CT data. We added 5% Gaussian noise in the numerical measurements. We have also investigated the effects of window size, neighborhood size and edge sensitivity parameters in kernel matrix on the reconstruction image quality. The results show that the spatial resolution and the accuracy have been improved significantly. The proposed method will be tested with agar phantom experiments. And we will compare our algorithm with the traditional soft prior based method.

10059-37, Session 8

Comparison of tomographic spectral and lifetime multiplexing in turbid media

Steven S. Hou, Brian J. Bacskai, Anand T. Kumar, Massachusetts General Hospital (United States)

Fluorescence lifetime and multispectral methods have commonly been used in microscopy to separately visualize multiple fluorophores in thin tissue samples using their unique fluorescence lifetime and spectral signatures, respectively. In microscopy, the separation of multiple spectral or lifetime signatures ("multiplexing") can be performed directly at each pixel using various linear or non-linear fitting techniques. However, in the case of thick turbid samples such as biological tissue, spectral or lifetime measurements at the surface of the sample are affected by light propagation through the turbid medium. Tomographic multiplexing in macroscopic samples therefore requires consideration of diffuse light transport in addition to the intrinsic spectra or lifetimes of fluorophores. Multispectral and lifetime imaging in diffuse media can be mathematically described in two steps, involving spectral or temporal mixing of fluorophores and diffuse light transport in the turbid medium. We show that the key difference between the two techniques is that the order of fluorophore mixing and diffuse propagation is reversed, resulting in a fundamental difference in their multiplexing capabilities, irrespective of measurement conditions. Using the resolution matrix to define a quantitative measure for inter-fluorophore cross-talk, we show that lifetime multiplexing, using the asymptotic time domain approach, provides zero cross-talk, while spectral multiplexing can achieve

zero cross-talk under special conditions. Simulations and experiments will be presented to compare the performance of spectral and lifetime multiplexing for the tomographic inversion of multiple fluorophores in complex shaped, heterogeneous turbid media.

10059-38, Session 8

A semi-learning algorithm for noise rejection: an fNIRS study on ADHD children

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In pediatrics studies, the quality of functional near infrared spectroscopy (fNIRS) signals is often reduced by motion artifacts. These artifacts likely mislead brain functionality analysis, causing false discoveries. While noise correction methods and their performance have been investigated, these methods require several parameter assumptions that apparently result in noise overfitting. In contrast, the rejection of noisy signals serves as a preferable method because it maintains the originality of the signal waveform. Here, we describe a semi-learning algorithm to detect and eliminate noisy signals. The algorithm dynamically adjusts noise detection according to the predetermined noise criteria, which are spikes, unusual activation values (averaged amplitude signals within the brain activation period), and high activation variances. Criteria were sequentially organized in the algorithm, and assessed signals were based on each criterion in order. By initially setting an acceptable rejection rate, particular criteria causing excessive data rejections are neglected, whereas others with tolerable rejections practically eliminate noises. fNIRS data measured during the attention response paradigm (oddball task) in children with attention deficit/hyperactivity disorder (ADHD) were utilized to evaluate and optimize the algorithm's performance. This algorithm successfully substituted the visual noise identification done in the previous study and consistently found significantly lower activation of the right prefrontal and parietal cortices in ADHD patients than in healthy children. Thus, we conclude that the semi-learning algorithm confers more objective and standardized judgment for noise rejection and presents a promising alternative to visual noise rejection.

10059-39, Session 8

Temporal and spatial blood flow changes quantified by diffuse correlation tomography predict the healing potentials of murine bone grafts

Songfeng Han, Ashley R. Proctor, Danielle S. W. Benoit, Regine Choe, Univ. of Rochester (United States)

Blood flow is important in bone graft healing, as blood supplies nutrients, oxygen, circulating cells and growth factors to the graft site, which are essential for healing. Non- invasive monitoring of blood flow in bone is difficult with current methods due to technical limitations (microspheres, laser Doppler) or cost concerns (MRI /PET). Diffuse correlation tomography (DCT) can non-invasively monitor three-dimensional blood flow in bone using near-infrared light. We have reported DCT revealed spatial and temporal blood flow differences among different mouse femoral graft approaches. Here, we present a strategy to build a predictive model for long-term mechanical strength of healed mouse femoral grafts based on temporal and spatial blood flow changes quantified by DCT.

To summarize the approach, our DCT system utilizes a galvo-based non-contact module to scan a source-detector pattern over tissue and obtain spatially dense data sets. For image reconstruction, a cost function based on Rytov approximation is minimized iteratively by building the Jacobian matrix and using Tikhonov regularization to stabilize the matrix inversion.

Weekly DCT measurements are performed on 3 groups of mice with autografts, allografts with and without tissue-engineered periosteum, from before graft transplantation to 9 weeks after. Micro-CT scans are performed to obtain mouse-specific finite element meshes for reconstruction. At 10 weeks after the graft surgery, healed femurs are harvested to test the mechanical strength, which is one of healing outcome measures. Finally, statistical analysis is performed to reveal the correlation between healing outcomes and blood flow derived parameters from the early weeks of healing.

10059-40, Session 9

Multi-wavelength photomagnetic imaging for breast cancer *(Invited Paper)*

Farouk Nouizi, Alex T. Luk, Michael Marks, Jaedu Cho, John Tu & Thomas Yuen Ctr. for Functional Onco-Imaging (United States); Seunghoon Ha, Philips Healthcare (United States); Hakan Erkol, Jessica Kwong, Gultekin Gulsen, John Tu & Thomas Yuen Ctr. for Functional Onco-Imaging (United States)

We have recently developed a new imaging technology, "Photo-magnetic Imaging" (PMI), which is capable of providing high resolution optical images. PMI is a unique and novel imaging technology that brings two modalities together: MR and Optical Imaging. Indeed, PMI is a true multimodality application: instead of working independently, both modalities work in a harmony to offer images that cannot be obtained by either one alone. PMI uses laser light to heat the medium under investigation but employ MRI to obtain temperature map of the tissue with high resolution. The MR temperature are then converted into the optical absorption maps using proper modeling of light propagation and heat transfer in tissue. Utilizing multiple wavelengths, photo-magnetic imaging can provide the same functional information as conventional optical imaging but with higher resolution due to utilization of MRI. The system uses only laser illumination, i.e. no optical detectors, so it will be a simple addition to clinical MR systems. The advantages of this new imaging modality are several-fold. First, it will allow measurements from the whole volume so that the inverse problem will not be underdetermined anymore. Utilizing multiple wavelengths, photo-magnetic imaging will provide the same functional information as conventional optical imaging but with much higher resolution. We will present the breast PMI interface, preliminary phantom and animal study results. We are currently applying for IRB approval to perform first human studies.

10059-41, Session 9

Diffuse reflectance spectroscopy as a tool for tumor detection in colorectal surgery: an ex vivo study

Elisabeth J. M. Baltussen, Susan G. Brouwer de Koning, Petur Snæbjörnsson, Koert F. D. Kuhlmann, Arend G. J. Aalbers, Niels F. M. Kok, Geerard Beets, Jasper Nijkamp, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (Netherlands); Bernardus H. W. Hendriks, Koninklijke Philips N.V. (Netherlands); Henricus J. C. M. Sterenborg, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (Netherlands) and Academisch

Medisch Centrum (Netherlands); Theo J. M. Ruers, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (Netherlands) and Univ. Twente (Netherlands)

Positive resection margins after colorectal surgery have a high negative prognostic value and are mainly caused by poor detection of tumor tissue embedded in pericolonic or mesorectal fat. With the use of fiberoptic diffuse reflectance spectroscopy (DRS), we want to provide real time assessment of the resection area to detect positive resection margins during surgery.

As a first step an ex vivo study was started, in which a total of 30 patients are included from whom tissue samples of tumorous tissue, healthy colon wall and fat from the mesocolon are obtained after resection. All samples are placed in a pathology cassette prior to the measurements. The locations of the measurements are carefully registered, to make it possible to match the measurements with pathology results. Measurements are performed using a DRS probe with source and detector fibers at fixed distances of 1 and 0.7 mm. Spectra are obtained in a wavelength range of 400-1600 nm. After normalizing the spectra at 800 nm, ROC curves are made to obtain the sensitivity and specificity for the distinction between tumor and fat.

Tumorous tissue can be distinguished from fat of the mesocolon in an ex vivo setting, with a sensitivity and specificity both higher than 0.95. Differences were measured mainly in the NIR range. These results show that DRS is a valuable technique for real time assessment of the resection area that eventually prevents positive resection margins. The next step will involve in vivo measurements to confirm the results in a less controlled situation.

10059-42, Session 9

Optical measurement of the effects of exercise training on muscle oxygen metabolism and blood flow in patients with peripheral artery disease

Wesley B. Baker, Zhe Li, Steven Schenkel, Malavika Chandra, David R. Busch, Erin K. Englund, Sarah J. Ratcliffe, Arjun G. Yodh, Univ. of Pennsylvania (United States); Thomas F. Floyd, Stony Brook Univ. (United States); Emile R. Mohler M.D., Univ. of Pennsylvania (United States)

Combining the optical techniques of near-infrared spectroscopy (NIRS) and diffuse correlation spectroscopy (DCS) permits study of deep tissue muscle oxygen metabolism and blood flow in patients with peripheral artery disease (PAD). Peripheral artery disease is most commonly caused by systemic atherosclerosis (i.e., narrowing of arteries by plaque) in the legs, and it affects approximately 8-10 million people in the United States. Many patients with PAD experience claudication, which is a walking-induced muscle pain that is relieved only with rest. Prior randomized clinical trials have demonstrated that supervised exercise training is an effective therapy for claudication. The mechanisms behind exercise-training induced improvement, however, are not well understood. We used frequency-domain NIRS and DCS to measure the maximal blood flow and oxygen metabolism increases in the calf muscle during treadmill exercise in 65 patients with peripheral artery disease. All patients were measured in an initial "pre-exercise visit", and 29 randomly selected patients then participated in a supervised three month exercise training program. After 3 months, all 65 patients came back for a second set of treadmill measurements. Exercise training increased maximal blood flow and oxygen metabolism by 67% (26%, 195%) and 75% (33%, 204%), respectively (results reported as median (25, 75 percentiles)). These increases are significantly different from the control group ($p < 0.001$). Our results suggest that improved endothelial vessel dilation of existing collateral blood vessels and improved muscle oxygen extraction are possible mechanisms for the effective treatment of claudication with exercise.

10059-43, Session 9

Optical measurement of blood flow in exercising skeletal muscle: a pilot study

Detian Wang, Institute of Fluid Physics (China); Wesley B. Baker, Ashwin B. Parthasarathy, Univ. of Pennsylvania (United States); Liguo Zhu, Zeren Li, Institute of Fluid Physics (China); Arjun G. Yodh, Univ. of Pennsylvania (United States)

Blood flow monitoring in skeletal muscle during exercise is important for sports medicine and muscle diseases. The optical technique of diffuse correlation spectroscopy (DCS) is a relatively new noninvasive way to monitor blood flow, but it is prone to artifacts from muscle fiber motion. Using a novel fast software correlator, we measured blood flow in forearm flexor muscles of N=2 healthy adults during handgrip exercise, at a sampling rate of 20 Hz. We simultaneously measured changes in muscle acceleration during exercise with an accelerometer. Combining the blood flow and acceleration data, we resolved the motion artifact in the DCS signal induced by muscle fiber motion, and isolated the blood flow component of the signal from the motion artifact. The results show that muscle fiber motion strongly affects the DCS signal, and if not accounted for, will result in an overestimate of blood flow (~1240% ± 600%). Our measurements indicate rapid dilation of arterioles following exercise onset, which enabled blood flow to increase to a plateau of 250% ± 40% in 13 s ± 2 s. The blood flow also rapidly recovered to baseline following exercise, i.e., 7 s ± 2 s.. Finally, preliminary results on the dependence of blood flow from exercise intensity changes will be discussed.

10059-44, Session 9

Diffuse optical tomography for in vivo 3D vascular imaging of a murine bone graft model

Jingxuan Ren, Haitong Wang, Ashley R. Proctor, Songfeng Han, Univ. of Rochester (United States); Jinchao Feng, Beijing Univ. of Technology (China) and Dartmouth College (United States); Scott C. Davis, Dartmouth College (United States); Regine Choe, Univ. of Rochester (United States)

Allografts are the current clinical gold standard for treating critical-sized bone defects. However, due to poor healing, allografts result in a 10-year post-implantation failure rate of 60%. Poor outcomes for allografts are partially due to poor host-mediated vascularization. Vascularization is, therefore, usually assessed to evaluate new treatments based on tissue engineering approaches for enhancing graft healing.

In this presentation, the applicability of diffuse optical tomography (DOT) for quantifying vascularization in a murine bone graft model is investigated. A frequency domain diffuse optical instrument was combined with a motorized stage based scanning module to develop a contact scanning DOT system. Phantom experiments were first conducted to test the quantification accuracy of the DOT system using a simplified tissue phantom capturing the main features of the mouse femur and the surrounding tissue. Then the left hindlimbs of five mice were measured before the allograft surgery and 1, 2, 3 weeks after the surgery, respectively. After the last DOT measurement, the mice were imaged using micro-CT to provide the distribution of the micro vasculature around the femur and the structural information of the hindlimb. Three-dimensional DOT reconstructions based on Near Infrared Fluorescence and Spectral Tomography (NIRFAST) are being conducted using the experimental data.

The preliminary DOT results from the pre-surgical measurements show a lower absorption coefficient in the region of the femur than in the region of the surrounding muscles, which is expected and matches the absorption coefficient values measured previously on the femur and on the muscle with diffuse optical spectroscopy.

10059-45, Session 9

Sentinel lymph node detection in gynecologic malignancies by a handheld fluorescence camera

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Near-infrared fluorescence imaging using indocyanine green (ICG) as a tracer is a promising technique for mapping the lymphatic system and for detecting sentinel lymph nodes (SLN) during cancer surgery. In our feasibility study we have investigated the application of a custom-made handheld fluorescence camera system for the detection of lymph nodes in gynecological malignancies. Our system comprises a low cost CCD camera with enhanced NIR sensitivity and two groups of LEDs emitting at wavelengths of 735 nm and 830 nm for interlaced recording of fluorescence and reflectance images of the tissue. The surgeon can observe fluorescent tissue structures overlaid onto the anatomical image on a monitor in real-time. The imaging system was applied for intraoperative lymphatic mapping in 5 patients with vulvar cancer, 5 patients with ovarian cancer, 3 patients with cervical cancer, and 3 patients with endometrial cancer. ICG was injected at four loci around the primary malignant tumor during surgery. After a residence time of typically 15 min fluorescence images were taken in order to visualize the lymph nodes closest to the carcinomas. For the cases with vulvar cancer about half of the lymph nodes detected by routinely performed radioactive SLN mapping have shown fluorescence in vivo as well. In the other types of carcinomas several lymph nodes could be detected by fluorescence during laparotomy. We conclude that our low cost camera system has sufficient sensitivity for lymphatic mapping during surgery.

10059-46, Session 9

Monitoring resistance training with diffuse optical spectroscopic imaging

Robert V. Warren, Univ. of California, Irvine (United States)

No Abstract Available

10059-47, Session 10

Non-invasive functional neuroimaging in the mouse using structured illumination diffuse optical tomography

Matthew D. Reisman, Zachary E. Markow, Adam Q. Bauer, Joseph P. Culver, Washington Univ. in St. Louis (United States)

The study of correlated spontaneous activity in functionally related brain regions using functional connectivity MRI (fcMRI) has allowed comprehensive mapping of distributed brain networks in humans. Although studies utilizing fMRI in mice have recently seen an increased application towards mapping resting state networks, the difficulty of obtaining high resolution images with sufficient signal to noise in mice using fMRI has led to optical intrinsic signal (OIS) techniques providing most of the observations of fc in the mouse brain. While effective, OIS requires scalp retraction and is limited to superficial cortical tissues. Diffuse Optical Tomography (DOT) provides non-invasive imaging, but current DOT systems are either too sparsely sampling to match the cortical resolution of OIS or are too slow for capturing spontaneous hemodynamics in the mouse brain. Here we develop a DOT system that combines the spatial sampling of camera-based systems with the rapid-imaging of structured illumination (SI) to non-invasively map activity in the mouse cortex.

The non-invasive mouse SI-DOT system is comprised of a sCMOS camera and a digital micromirror device (DMD) that allow for rapid, flexible illumination and detection with high spatial resolution. By expanding the system to include multiple cameras detecting from multiple views, we can greatly improve our depth sensitivity with simultaneous reflectance and transmission measurements. Custom data quality assessment metrics allow for measurement optimization, harnessing the system's complete flexibility of wavelengths, illumination patterns, and detector binning to provide a powerful framework for 3D mapping of mouse brain networks.

10059-48, Session 10

Bedside mapping of brain function during acute stroke recovery using high-density diffuse optical tomography

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The first 72 hours after an ischemic stroke, typically triggered by an occlusion of an artery in the brain, can be highly unstable. For an optical neuroimaging tool to provide near-real-time bedside monitoring that can assess changes in neurological status and potentially inform clinical decisions, the system must yield adequate data quality over a field of view that encompasses multiple functional domains. To address these challenges, we developed a portable high-density DOT system that contains a field of view covering sensory, motor, and cognitive brain regions along with data quality metrics such as real-time cap coupling and automated motion artifact detection. To establish that the portable HD-DOT system is sensitive to disruption in brain function, we collected up to an hour of resting state DOT data from 44 patients within the first 72-hours post stroke. The NIH Stroke Scale NIH-SS provided a behavioral metric of stroke-induced functional deficit. Standard DOT functional connectivity analyses (fcDOT) were performed on the [HbO2] data using regions of interest (ROI) located at every voxel within the field of view. For each ROI-based fc map, the spatial correlation (called 'similarity') was calculated between the fc maps of the stroke patient and a healthy population. The distribution of similarity values across the entire field of view reveals a skewness that is significantly correlated with NIH-SS, $p<7e-4$. Herein, we have demonstrated that HD-DOT is sensitive to altered brain function brought about by ischemic stroke as measured within the first 72 hours of stroke onset.

10059-49, Session 10

Measuring cerebral blood flow with near-infrared spectroscopy

Kristen T. Tgavalekos, Angelo Sassaroli, Sergio Fantini, Tufts Univ. (United States)

We used a mathematical hemodynamic model and measurements of cerebral oxyhemoglobin (O) and deoxyhemoglobin (D) concentrations from near-infrared spectroscopy (NIRS) to compute absolute values and dynamic changes in cerebral blood flow. The model analytically relates O and D to cerebral blood flow (CBF), cerebral blood volume (CBV), and the cerebral metabolic rate of oxygen (CMRO2). In order to compute CBF, a number of parameters including the blood transit time in the vasculature, the blood volume contributions from the various vascular compartments,

and the efficiency of autoregulation were determined by fitting measured coherent changes in O and D to the model expressions. Our approach for inducing coherent cerebral O and D changes in human subjects is with a two minute arterial occlusion in both thighs with pneumatic cuffs followed by a rapid release, which causes a transient decrease in systemic mean arterial blood pressure. The cerebral blood flow velocity response to this thigh cuff occlusion and release maneuver has been well characterized with transcranial Doppler ultrasound (TCD). We compared our results for microvascular dynamic CBF obtained with NIRS and the hemodynamic model with previous results obtained with TCD. We found that, similar to the TCD results, CBF recovery precedes arterial blood pressure recovery in healthy subjects with intact autoregulation. We also compared our measurements of CBF with diffuse correlation spectroscopy (DCS) during a breath holding protocol, finding a good qualitative agreement. Our proposed approach to NIRS-based measurements of CBF has potential as a blood flow monitoring tool in a clinical setting.

10059-50, Session 10

Spatial distribution of induced hemodynamic oscillations in superficial, extracerebral tissue for coherent hemodynamics spectroscopy

Angelo Sassaroli, Xuan Zang, Kristen T. Tgavalekos, Sergio Fantini, Tufts Univ. (United States)

Brain studies with functional near-infrared spectroscopy (fNIRS) are affected by hemodynamic changes occurring in superficial extracerebral tissue (scalp, skull, etc.). Several studies have addressed the spatial uniformity of these changes when they are originated from spontaneous fluctustions or systemic effects of brain stimulation paradigms. If superficial hemodynamic changes are spatially uniform, one can apply cancellation algorithms based on measurements taken at a short source-detector separation in order to retrieve the brain hemodynamics from data at a long source-detector separation. This method has been previously proposed and applied to measuring cerebral hemodynamic changes associated with neuronal activation. By contrast, coherent hemodynamics spectroscopy (CHS), which is a novel method to study microcirculation integrity, is based on inducing coherent hemodynamic oscillations of oxy- and deoxyhemoglobin concentrations in tissue. The oscillations can be induced by different forcing mechanisms such as paced breathing or (in this study) cyclic thigh cuff occlusions and releases. We present experimental results about the spatial distribution of induced oscillations in scalp/skull tissue. By using a source-detector arrangement that allows for two short (~5 mm) separations, in addition to the standard (~30 mm) separation for fNIRS, we are able to test if induced superficial oscillations may be considered to be uniform in the extracerebral tissue. If not, both short separation channels must be used to correct CHS data in order to identify the cerebral hemodynamic oscillations. Such induced cerebral hemodynamics reflect relevant physiological parameters, including capillary and venous blood transit times, cerebral autoregulation, and microvascular blood volume.

10059-51, Session 11

Validation of diffuse optical spectroscopic measurement of cerebral oxygen metabolism in a piglet model of deep hypothermic circulatory arrest (DHCA)

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Introduction: Deep hypothermic circulatory arrest (DHCA) is an important, neuroprotective strategy wherein the patient is cooled below 20˚C via cardiopulmonary bypass (CPB) to stabilize cerebral metabolism while infants and children undergo surgical repair of congenital heart disease (CHD) (Ziganshin, 2013; Erecinska, 2003). Despite widespread use over four decades, uncertainty remains per the optimal management of DHCA protocols for cooling and rewarming for prevention of hypoxic-ischemic-reperfusion brain injury (Greeley, 1991; Oates, 1995; Wypij, 2003).

Methods: Neonatal piglets (3.5-4.0 kg) undergo identical anesthetic, surgical, CPB, and DHCA protocols translated from human neonatal cardiac procedures. Our primary outcome is the validation of continuous, non-invasive, frequency-domain diffuse optical spectroscopy (FD-DOS) and diffuse correlation spectroscopy (DCS) measurements of cerebral oxygen metabolism and cerebral blood flow compared to invasive measurements of intracranial oxygen content (Integra Licox) and cerebral perfusion (Perimed AB). Secondary outcome measures include: intracranial temperature (Licox, Integra Life Sciences), subcortical microdialysis sampling of injury and metabolism markers (CMA Microdialysis AB) and bilateral electroencephalograms (EEG) (Subdermal Needle Electrodes, Rhythmlink), compliling a comprehensive, multi-modal assessment of cerebral pathophysiology.

Interim Results and Conclusions: Non-invasive measures of cerebral hemodynamics trend with invasive measurements during circulatory arrest and reperfusion (n=8). Development and validation of a non-invasive, continuous neuromonitoring device has been a critical barrier to understanding pathologic alterations of cerebral metabolism during CPB and DHCA, and ultimately optimizing neuroprotective strategies to the individual patient. Our data provides initial proof of concept that FD-DOS and DCS may overcome this barrier for CHD neonates as well as other vulnerable patient populations.

10059-52, Session 11

A fast atlas-guided high density diffuse optical tomography system for brain imaging

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Near infrared spectroscopy (NIRS) is an emerging functional brain imaging tool capable of assessing cerebral concentrations of oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (HbR) during brain activation non-invasively. As an extension of NIRS, diffuse optical tomography (DOT) not only shares the merits of providing continuous readings of cerebral oxygenation, but also has the ability to provide spatial resolution in the millimeter scale. Based on the scattering and absorption properties of nonionizing near-infrared light in biological tissue, DOT has been successfully applied in the imaging of breast tumors, osteoarthritis and cortex activations. Here, we present a fast high density DOT system with a state-of-art machine-brain interface, which is suitable for preclinical or clinical brain imaging applications. It can achieve up to a 21 Hz sampling rate for a full set of two-wavelength data for 3-D DOT brain image reconstruction. The system was validated using tissue-mimicking brain-model phantom. Then, cognitive-task experiments on healthy subjects were conducted to demonstrate the capability of the system.

10059-53, Session 11

A compact dual wavelengths time-domain fNIRS system

Davide Contini, Mauro Buttafava, Edoardo Martinenghi, Alberto Dalla Mora, Antonio Pifferi, Alberto Tosi, Alessandro Torricelli, Politecnico di Milano (Italy)

We proposed a novel compact time domain fNIRS system based on solid state technology. We developed a one-channel system with two wavelengths in order to estimate the hemoglobin content in tissue, in particular the brain cortex. The system is very compact (16x22x5 cm). As pulsed light source, we opted for two laser diodes operating in gain switching and thermalized by means of a thermoelectric cooler. The two laser sources emit pulse trains at 685 nm and 830 nm with a repetition frequency of 40 MHz, an average power of 0.3 mW and 2.5 mW respectively and a FWHM of the single pulse around 200 ps. The collected light is focused by a proper optics on a silicon photomultiplier (SiPM) of 1 mm of diameter (C30742-11-050-T1, Excelitas) with a detection efficiency of around 15%. The detection module shows a time response of about 100 ps and a photon noise level of around 90 kcps. The acquisition electronics is based on a Time to Digital Converter chip developed by Politecnico di Milano that shows the following characteristics: 10 ps of channel width, 40 ps FWHM single shot precision and 3.5 Mconv/s of maximum conversation rate. The system is suitable for oxygen saturation determination in tissue in particular for brain monitoring. The compactness of this device and the possibility to be battery operated (power consumption is < 10 W) pave the way to numerous applications, even for developing countries. Fully phantom characterization and first in vivo applications will be also presented.

10059-54, Session 11

Cerebral hemodynamic responses to hypocapnia and hypercapnia: multi-wavelength time-resolved NIRS studies

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We applied a time-resolved NIRS instrument in measurements of diffuse reflectance at multiple wavelengths simultaneously in order to investigate the cerebral hemodynamic changes during controlled hypo- and hypercapnia. The optodes were positioned above a forehead on a head of a healthy volunteer. The instrument was used to record distributions of times of flight of photons (DTOF's) at single source–detector separation of 3 cm during normocapnia, hypocapnia (-15mmHg) and hypercapnia (+15mmHg). The recorded DTOF's were analyzed by calculating their statistical moments (the number of diffusely reflected photons N, the mean time of flight of photons < t>, and the variance V of DTOF) for 16 wavelengths from near-infrared region (from 650 to 850nm with the step of 12.5 nm). The calculated moments show a different sensitivity to changes in absorption in tissue at different depths.

In the hypocapnia test, after a 1 minute rest (normocapnia) period, a decrese in the total number of photons was observed after beginning of test. Respectively, an increase in the N in the case of hypercapnia was noted. In the higher-order statistical moments of DTOFs, it was observed that after sudden changes in the signals, they tenden to return to their initial values until the change of end tidal carbon dioxide reached its maximum value. The estimated concentration changes of oxy-, deoxy- and total hemoglobins shows a similar pattern of changes as the high-order moments od the DTOFs. Implementation of the method based on moments of DTOFs ensures that the monitored changes in the hemoglobin concentrations reflect the changes in oxygenation occurring in the intracerebral tissue layers.

10059-55, Session 11

Noninvasive Monitoring of Cerebral Autoregulation with Diffuse Correlation Spectroscopy in Brain-injured patients

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Patients after traumatic brain injury may suffer from impaired cerebral autoregulation (CA), which inhibits the brain to maintain relatively constant blood flow regarding changes in perfusion pressure. Understanding the complex pathophysiology of these injuries is critical to develop improved prognostic and therapeutic approaches. Multimodal brain monitoring (MMM) has evolved over the last several years as a tool to understand the mechanisms of brain injury and brain function. It provides continuous measurement of brain-tissue oxygenation, regional and global blood flow (CBF) and microdialysis. In this study we used diffuse correlation spectroscopy (DCS) to noninvasively measure CBF following injection of Nicardipine, which was used to reduce blood pressure. We investigated relationships between CBF and other MMM values to provide insights into function that occurs in the brain injury. Relative CBF (rCBF) denoted as the CBF percentage change relative to its baseline was calculated and compared with mean arterial pressure (MAP) and pressure reactivity index (PRx). Following drug injection, MAP dropped from 113.6 mmHg to 87.8 mmHg (22.7% drop) and rCBF dropped 22.6% from its peak value. The observed linear relationship between rCBF and MAP demonstrates impaired CA (R2=0.96, slope = 1.03 %CBF/mmHg). PRx was calculated as a moving Pearson correlation coefficient between MAP and intracranial pressure (ICP), which is invasively measured. Positive correlation between MAP and ICP at lower frequency was found indicating passive cerebral vasculature and impaired CA, which is in agreement with the noninvasive DCS assessment of CA. DCS probes are well-suited for monitoring CA over prolonged periods of time and can be secured anywhere on the head.

10059-56, Session 12

The changes of cerebral hemodynamics during ketamine induced sedation in a rat model

Jay Young Bae, Seonghyun Kim, Dong-Hyuk Choi, Dongrae Cho, Jinsil Ham, Boreom Lee, Jae Gwan Kim, Gwangju Institute of Science and Technology (Korea, Republic of)

Ketamine is a NMDA receptor antagonist and shows very distinctive features among other anesthetic agents. Ketamine is the only intravenous anesthetic agent which has excitatory CNS effects. Owing to this difference, existing EEG-based anesthetic depth monitoring method cannot be applied to ketamine. In this study, we employed a near-infrared spectroscopy (NIRS) to observe cerebral hemodynamic changes induced by ketamine in rats to see if NIRS can be used to monitor the depth of sedation non-invasively in a real-time. Our system consists of a tungsten-halogen lamp, a NIR range spectrometer, a computer and a pair of fiber-optic patch cables. A spectrum in the range of 730-850 nm were acquired at 3 Hz. In order to measure the NIR signals from the brain, we implanted optodes into two positions (Right/Left frontal cortex) on the skull of the rat. We acquired EEG and NIRS signals 30 minutes from freely moving rats as a baseline. Afterwards rats were administered ketamine by intravenous route, and the acquisition of EEG and NIRS signals were continued during the sedation and until the rat

awakes. The acquired intensity values at 5 wavelengths (730, 750, 800, 830, 850 nm) were used to estimate the concentration of deoxy-, oxy- and total hemoglobin changes in rat brain by using the modified Beer-Lambert's law. These hemodynamic changes were compared with the EEG signals. From this study, cerebral hemodynamic changes in rats were shown during the ketamine injection. Further investigations are needed to characterize the factors that affect hemodynamics.

10059-57, Session 12

Frontal hemodynamic changes during photothrombotic ischemia using diffuse reflectance spectroscopy

Seonghyun Kim, Sungchul Kim, Ji-Young Park, Hyoung-Ihl Kim, Jae Gwan Kim, Gwangju Institute of Science and Technology (Korea, Republic of)

Despite the mechanism of diaschisis is very important to know, it is not yet fully understood. PET or fMRI imaging technique may provide key answers to a question of how diaschisis occurs. However, a longitudinal study to monitor how ischemic stroke is connected with diaschisis occurrence is not easy to perform using aforementioned systems. This study aims to find differences between pre- and post-stroke state in terms of frontal hemodynamics using diffuse optical spectroscopy which will be the first step to find a connection between ischemic stroke and diaschisis. In order to measure frontal hemodynamics, we implanted 4 optodes on the frontal cortex part of the brain (8 weeks old, Sprague-Dawley Rats). An optical fiber was stereotactically inserted into the internal capsule (IC) target for photothrombosis. Rose Bengal dye was injected through a tail vein and photothrombotic destruction of the IC was conducted with 90 sec of 532 nm laser irradiation (2.5mW). As it is forming photothrombosis at intended area, we acquired optical spectrum to monitor cerebral hemodynamics. The concentrations of the oxy-, deoxy-, and total hemoglobin were estimated by fitting optical spectrum with a mathematical model derived from diffusion theory. Diffuse reflectance spectroscopy may provide the degree of ischemic stroke and guide a treatment strategy by providing the efficacy of treatment.

10059-58, Session 12

The monitoring of cerebral oxygenation with a variation of isoflurane concentration in a rat model

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In clinical practice, estimating the depth of anesthesia (DOA) is important for monitoring safety during surgical treatment. Anesthetic depth indicators based on electroencephalography (EEG) are commercially available. However, this may give rise to erroneous values in patients with abnormal EEG. Therefore, we aimed to investigate experimentally how anesthetic levels affect cerebral metabolism measured by near-infrared spectroscopy (NIRS) and identify a robust marker among NIRS parameters to discriminate various stages of anesthetic depth in rats under isoflurane anesthesia.

In order to record the hemodynamic changes and local field potential (LFP) in the brain, fiber-optic cannulas and custom-made microelectrodes were implanted in the frontal cortex of the skull. The rats were anesthetized with decreasing isoflurane concentration from 2.5% to 1% with a step of 0.5% every 5 minutes. The hemodynamic changes from NIRS and LFP signals were continuously monitored at each isoflurane concentration level.

As a result of our experiments, level of oxyhemoglobin and total hemoglobin concentration of the frontal cortex decreased gradually as isoflurane concentration is reduced, but deoxyhemoglobin (RHb) increased. The reflectance ratio between 730nm and 850nm and burst suppression ratio (BSR) correspond similarly with the change of oxyhemoglobin during the variation of isoflurane concentration. These results suggest that NIRS signals in addition to EEG may provide a possibility of developing a new anesthetic depth index. It is expected that the results of this study can be used as a reference when using a non-invasive way of future research.

10059-59, Session 12

Influence of intracranial pressure on cerebral hemodynamic changes measured with near infrared spectroscopy

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Cerebral autoregulation (CA) is a mechanism that maintains consistent blood supply towards the brain despite changes in cerebral perfusion pressure (CPP), which is defined as the difference between mean arterial blood pressure (MAP) and intracranial pressure (ICP). Since this mechanism is impaired in many diseases, including traumatic brain injuries and stroke, abilities of monitoring CA are important. One way of quantifying CA is to calculate the moving correlation between ICP and MAP, known as the pressure reactivity index (PRx). This index describes how well the brain is adapting to MAP changes, where negative correlation to ICP is indicative of autoregulation being intact. However, the PRx index assumes that MAP changes trigger ICP changes. The reverse, how ICP changes influence MAP changes, is not well understood. In addition, non-invasive alternatives to ICP monitoring are needed.

In order to address this need and question, we performed experiments on non-human primates (n=4). We measured cerebral hemodynamic changes, i.e. changes in oxygenated and deoxygenated hemoglobin concentration, with a frequency domain NIRS system (OxiplexTS, ISS Inc.). The primates' brains were cannulated to induce rapid changes in ICP and the response in MAP and NIRS signals were recorded. Our results indicate a high correlation between CPP and total hemoglobin concentration changes, which demonstrates the ability of NIRS to substitute ICP without the need of invasive monitors. We will further present results from this NIRS based approach for quantifying CA as well as the analytical model used for analysis, in cases where ICP changes lead MAP changes.

10059-60, Session 12

Assessments of cerebral autoregulation in extracorporeal support based on wavelet transform coherence (WTC)

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Extracorporeal membrane oxygenation (ECMO) is a form of advanced cardio-respiratory support provided to critically ill patients with severe respiratory and/or cardiovascular failure. It is typically used as a rescue therapy for many life-threatening, reversible conditions such as severe shock states, severe respiratory failure, congenital heart disease and neonates with persistent pulmonary hypertension. ECMO therapy is associated with significant mortality and morbidity, which are attributed to a number of

pre-ECMO and ECMO-related factors such as severe hypoxia, hypercarbia, hypertension and cannulation of great blood vessels. Currently there is no reliable tool at the bedside to evaluate the neurological events during ECMO. Diagnostic imaging such as CT scans are often too challenging for these patients.

In this study, we assessed the cerebral autoregulation in patients during extracorporeal support (N = 27) and explored its potential role for developing cerebral injuries. In particular, wavelet transform coherence (WTC) was applied to quantify the nonstationary cross-correlation between mean arterial pressure (MAP) and cerebral tissue oxygen saturation (SctO2). Our results show impaired cerebral autoregulation is an early indicator of acute cerebral injuries which were seen on CT and/or MRI post to ECMO. Thus, non-invasive monitoring of the cerebrovascular impairments during ECMO may be a key for modifying ECMO factors and enabling early interventions in order to improve treatment outcome.

10059-61, Session 12

Spectroscopic optical microangiography (SOMAG)

Woo June Choi, Kwanseob Park, Ruikang K. Wang, Univ. of Washington (United States)

The mammalian brain is an aerobic organ that depends on a continuous and adequate supply of oxygen to maintain its structural and functional integrity. The cerebral metabolic rate of oxygen consumption (CMRO2) is one of the key parameters to describe normal brain functions. The determination of CMRO2, however, requires measurements of blood oxygenation, blood flow velocity, and vessel morphology. In this preliminary study, we propose the feasibility of functional optical microangiography (OMAG) to quantify blood oxygenation in the functional vessels. We show that it is feasible to estimate saturation oxygen (SaO2) levels in the blood with the spectral OMAG signals measured using a single broadband light source that covers the isosbestic absorption point at 805 nm.

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10060-1, Session 1

Accuracy of reflectance confocal microscopy for diagnosing skin lesions in vivo in real-time at the bedside: understanding challenges and potential pitfalls *(Invited Paper)*

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Introduction: Reflectance confocal microscopy (RCM) non-invasively images skin lesions in vivo at cellular resolution and can guide management of patient care. While previous studies have retrospectively demonstrated the high accuracy of RCM in diagnosing skin lesions, this study prospectively assessed the potential of RCM in clinical settings for diagnosis in real-time at the bedside.

Methods: 127 skin lesions were imaged and diagnosed to determine BCC in non-melanocytic lesions (NML) and melanoma in new/growing melanocytic lesions (ML). Most of these lesions had an equivocal diagnosis on dermoscopy.

Results: Histopathological diagnosis was available in 78/127 (61.4%) lesions, including 50 NMLs (24 BCCs, 3 SCCs and 23 benign) and 28 MLs (6 melanomas, 22 nevi). The remaining 49/127 lesions (38.6%) were not biopsied (5 received topical treatment, 44 monitoring). On RCM, 20/24 (83.3%) BCCs and 4/6 (66.6%) melanomas were correctly diagnosed. BCC was missed in 3/24 (12.5%) lesions and melanoma in 1/6 (16.6%) lesions; these were diagnosed as superficial BCCs and focal epidermal changes overlying deeply situated melanoma nodule on histopathology, respectively. False positive diagnosis of BCC was obtained in 7/23 (30.4%) lesions and of melanoma in 1/22 (4.5%) lesions; these were diagnosed mostly inflammatory and moderately atypical dysplastic nevus on histopathology, respectively. In 7 lesions BCC and melanoma could not be ruled out definitely. We obtained diagnostic accuracy of 80.28% with high sensitivity and specificity of 80.68% and 80.8%, respectively in differentiating benign from malignant lesions.

Conclusion: RCM can differentiate benign from malignant skin lesions in vivo with high accuracy in real-time at the bedside. However, some pitfalls must be addressed to improve diagnostic accuracy and thus reduce the rate of unnecessary biopsy.

10060-2, Session 1

Visualization of skin cancer using hyperspectral imaging *(Invited Paper)*

Larisa A. Zherdeva, Ivan A. Bratchenko, Oleg O. Myakinin, Samara Univ. (Russian Federation); Alexander A. Moryatov, Sergey V. Kozlov, Samara State Medical Univ. (Russian Federation); Valery P. Zakharov, Samara Univ. (Russian Federation)

Study results of hyperspectral imaging for in vivo visualization of skin neoplasms are presented. The melanoma, basal cell carcinoma, pigmented nevi and benign with different stages of neoplasm growth are investigated. The ethical committee of Samara State Medical University approved the protocol of tissues study. The final diagnosis of studied samples has been defined on the basis of histological analysis.

The system of hyperspectral imaging can display the images with spectral resolution of up to 2.1 nm in the range of 450-750 nm and provides the capability to obtain images at a rate of 3 frames per second with a resolution of 1360x1024 pxl (maximum increase in scanning area is 7x7 cm). The obtained data has been processed in MATLAB R2014a.

Prior to analysis of hypercube data, a frame at each wavelength has been stabilized during the scanning time interval relative to a selected stationary frame. This step is necessary due to a displacement of the scanning area related with spontaneous macro-movements of the patient (breathing, for instance). Backscattering spectra are stimulated by white LED source. Also, 457 nm laser are selected as the excitation source of autofluorescence.

As a result, the index of optical densities amounts in maximum of hemoglobin and melanin absorption ranges (530-600 and 600-670 nm respectively) and the distribution of index values in the entire neoplasm area allows to differentiate the type of pathological tissues.

10060-3, Session 1

Confocal imaging-guided laser ablation of basal cell carcinomas: initial in vivo results *(Invited Paper)*

Heidy Sierra, Miguel A. Cordova, Oriol Yelamos, Anthony Rossi, Chih-Shan Jason Chen, Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Surgery is the standard treatment for Basal cell carcinomas (BCCs). However, nonsurgical approaches are being increasingly preferred for lower risk BCCs, to avoid excess tissue removal and to improve recovery time and cosmetic outcome. Laser ablation can be less invasive, less expensive and faster than surgery. However, the lack of histopathological confirmation post-ablation produces variable efficacy and has been a limitation for routine implementation in the clinic.

Based on previous ex vivo and initial in vivo testing studies, a reflectance confocal microscopy (RCM) imaging-guided laser ablation methodology is being developed: RCM is used to detect tumor margins before ablation with a pulsed Er:YAG (250 ?sec, 25 J/cm2, 1-6 passes) or CO2 (750 ?sec, 7.5 J/cm2, 1-4 passes) laser. The wound is swabbed with 35% AlCl3 to enhance nuclear contrast, at the peripheral epidermal and the central portion of the deep dermal margins and then imaged to provide histopathology-like feedback.

Forty superficial and nodular BCCs have been treated with image-guided laser ablation and being followed-up with additional imaging. Two Mohs surgeons evaluated post-ablation images for the presence or absence of tumor and consistency of the uptake of AlCl3 to label nuclei. Initial results show saturation artifacts in the imaging of two out of 10 lesions. Post-treatment follow-up imaging is being performed at 3, 6 and 18 months for detection of any recurrent tumor, wound healing and evaluation of cosmetic outcome. Imaging showed clearance of BCCs in all lesions for which 15 lesions have been imaged at 3 months, 16 at 3 and 6 months and 14 at 3, 6 and18 months.

Further investigation and optimization to image over the entire wound (without missing any areas) and to enhance tumor-to-dermis contrast for clinically acceptable sensitivity and specificity is needed.

10060-4, Session 1

In vivo confocal Raman spectroscopic analysis of the effects of IR radiation in the stratum corneum of human skin *(Invited Paper)*

Ramu Rajasekaran, Monica Bergamo Lopes, Taciana

D. Magrini, Ana Clara Figueira Lopes Cançado, Airton Abrahao Martin D.D.S., Univ. do Vale do Paraíba (Brazil)

Stratum Corneum is the outer covering of the body, which serves as a barrier to infection. The composition of the skin changes withexternal environmental factors, such as temperature, sun irradiation, air pollutants, chemical hazards, as well as other factors.Solar radiation,especially IR radiation is being used as medicine for wound healing processes, in cosmetology, in physiotherapy and warming of muscles. Also, it was reported that the IR radiation produces free radicals and the excess production of free radicals causes irreversible damages. It has been reported that heat may be transmitted by IR radiation, which results in raised skin temperature and the chronic heat exposure of human skin may cause alterations. Erythema igne is one such disease known to be caused by chronic heat exposure. Many techniques have been adopted for monitoring the changes in the skin, which includes the tape stripping and biopsy as the primary methodology. However, these in vitro techniques are invasive, time consuming, and may not provide the actual information as in in vivo conditions. Confocal Raman spectroscopy,which is non-invasive and real time was considered as a potential tool for the in vivo analysis of the distribution and characteristics of different metabolic conditions and their variations of the skin. In this regard, we aimed at in vivo characterization of the IR induced changes in the stratum corneum of human volunteers. The results of Raman spectral signatures with respect to the control and IR exposed skin will be discussed.

10060-5, Session 1

Performance of mid infrared spectroscopy in skin cancer cell type identification *(Invited Paper)*

Lena Kastl, Björn Kemper, Westfälische Wilhelms-Univ. Münster (Germany); Gavin R. Lloyd, Univ. of Gloucestershire (United Kingdom); Jayakrupakar Nallala, Nick Stone, Univ. of Exeter (United Kingdom); Valery Naranjo, Francesco Penaranda, Univ. Politècnica de València (Spain); Jürgen Schnekenburger, Westfälische Wilhelms-Univ. Münster (Germany)

Marker free optical spectroscopy is a powerful tool for the rapid inspection of pathologically suspicious skin lesions and the non-invasive detection of early skin tumors. This goal can be reached by the combination of signal localization and the spectroscopical detection of chemical cell signatures. We here present the development and application of mid infrared spectroscopy (midIR) for the analysis of skin tumor cell types and three dimensional tissue phantoms towards the application of midIR spectroscopy for fast and reliable skin diagnostics. We developed standardized in vitro skin systems with increasing complexity, from single skin cell types as fibroblasts, keratinocytes and melanoma cells, to mixtures of these and finally three dimensional skin cancer phantoms. The cell systems were characterized with different systems in the midIR range up to 12 ?m. The analysis of the spectra by novel data processing algorithms demonstrated the clear separation of all cell types, especially melanoma cells. Special attention and algorithm training was required for closely related mesenchymal cell types as dedifferentiated melanoma cells and fibroblasts. Proof of concept experiments with mixtures of in vivo fluorescence labelled skin cell types allowed the test of the new algorithms performance for the identification of specific cell types. The intense training of the software systems with various samples resulted in a increased sensitivity and specificity of the combined midIR and software system. These data highlight the potential of midIR spectroscopy as sensitive and specific future optical biopsy technology.

10060-6, Session 2

Toward intravascular morphological and biochemical imaging of atherosclerosis with optical coherence tomography (OCT) and fluorescence lifetime imaging (FLIM) *(Invited Paper)*

Xi Chen, Wihan Kim, Michael Serafino, Texas A&M Univ. (United States); Brian Walton, The Univ. of Texas Health Science Ctr. at Houston (United States); Javier A. Jo, Brian E. Applegate, Texas A&M Univ. (United States)

We have shown in an ex vivo human coronary artery study that the biochemical information derived from FLIM interpreted in the context of the morphological information from OCT enables a detailed classification of human coronary plaques associated with atherosclerosis. The identification of lipid-rich plaques prone to erosion or rupture and associated with sudden coronary events can impact current clinical practice as well as future development of targeted therapies for “vulnerable” plaques. In order to realize clinical translation of intravascular OCT/FLIM we have had to develop several key technologies. A multimodal catheter endoscope capable of delivering near UV excitation for FLIM and shortwave IR for OCT has been fabricated using a ball lens design with a double clad fiber. The OCT illumination and the FLIM excitation propogate down the inner core while the large outer multimode core captures the fluorescence emission. To enable intravascular pullback imaging with this endoscope we have developed an ultra-wideband fiber optic rotary joint using the same double clad fiber. The rotary joint is based on a lensless design where two cleaved fibers, one fixed and one rotating, are brought into close proximity but not touching. Using water as the lubricant enabled operation over the near UV-shortwave IR range. Transmission over this bandwidth has been measured to be near 100% at rotational frequencies up to 147 Hz. The entire system has been assembled and placed on a mobile cart suitable for cath lab based imaging. System development, performance, and early ex vivo imaging results will be discussed.

10060-7, Session 2

Optical biopsy of coronary atherosclerotic plaques with intravascular polarimetry *(Invited Paper)*

Martin Villiger, Wellman Ctr. for Photomedicine (United States); Antonios Karanasos M.D., Erasmus MC (Netherlands); Pallavi Doradla, Wellman Ctr. for Photomedicine (United States); Kenichiro Otsuka, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Jian Ren, Norman Lippok, Milen Shishkov, Wellman Ctr. for Photomedicine (United States); Gijs van Soest, Erasmus MC (Netherlands); Peter Libby, Brigham and Women’s Hospital (United States); Evelyn Regar M.D., Erasmus MC (Netherlands); Seemantini K. Nadkarni, Brett E. Bouma, Wellman Ctr. for Photomedicine (United States)

Myocardial infarction, caused by the rupture of an unstable coronary plaque, remains the leading cause of death in the developed world. The development of improved therapies relies on detailed understanding of the pathogenesis of atherosclerosis and ensuing thrombotic complications. Optical biopsy with intravascular optical coherence tomography (OCT) provides one of the only means to study the microstructure of atherosclerotic plaques in human patients. There prevails a need for additional contrast to dissect individual aspects of plaque morphology, such as collagen and smooth muscle cell content, macrophage accumulation, and the content of cholesterol crystals. We developed intravascular polarimetry to explore how individual plaque components alter the polarization of

infrared light. Based on polarization sensitive optical coherence tomography, we detect the polarization state of the light scattered by subsurface microstructures. Advanced reconstruction strategies correct for polarization distortions induced by system components and the rotating catheter. After completing an ex vivo imaging study on cadaveric hearts for correlation with histology, we performed a clinical pilot study in 30 patients. Tissue rich in collagen and smooth muscle cells exhibited elevated birefringence. The optic axis orientation offered additional insight into the physical orientation of collagen fibrils. Furthermore, regions rich in lipids, cholesterol crystals, and macrophages displayed depolarization. These are important aspects of the mechanical integrity and vulnerability of atherosclerotic plaques, and may help in identifying high-risk plaques. Intravascular polarimetry could provide a new means to evaluate the effects of therapeutic interventions and potentially enable innovative approaches to personalized diagnostics in the clinic.

10060-8, Session 2

Clinical experience with spectrally encoded confocal microscopy for imaging human esophagus in vivo *(Invited Paper)*

DongKyun Kang, Massachusetts General Hospital (United States); Nima Tabatabaei, York Univ. (Canada); Dukho Do, Guillermo J. Tearney, Massachusetts General Hospital (United States)

Diagnosis of esophageal diseases is often hampered by tissue sampling errors associated with standard endoscopic biopsy. Spectrally encoded confocal microscopy (SECM) is a high-speed reflectance confocal microscopy technology that has the potential to image the entire esophagus at microscopic resolution and significantly reduce the tissue sampling errors. We have developed three distinctive SECM endoscopic devices and have utilized them to image patients with Barrett's esophagus (BE) and eosinophilic esophagitis (EoE). In this paper, we present preliminary findings from SECM clinical studies and discuss remaining challenges.

10060-54, Session 2

Diffraction limited mid-infrared spectroscopy with a supercontinuum laser source

Laure Lavoute, NOVAE (France); Christophe L. Sandt, Ferenc Borondics, Synchrotron SOLEIL (France); Nicolas Ducros, NOVAE (France); Sébastien Février D.D.S., NOVAE (France) and XLIM Institut de Recherche (France)

FTIR spectromicroscopy in the mid infrared region reveals the chemical composition of materials. At the highest performance, synchrotron sources provide diffraction limited spatial resolution. Powerful broadband light sources with continuous spectral power density in the mid infrared and diffraction-limited beam characteristics would complement the synchrotron source. In this communication, we explore the potential of a mid-infrared all-fiber supercontinuum laser as an alternative to the synchrotron source for spectromicroscopy of biological tissues.

Broadband light generation can be achieved by exalting cubic nonlinearities in fibers pumped in the anomalous dispersion regime by means of short pulse lasers. We developed a turn-key supercontinuum laser covering the spectral region from 1.9 to 4 μm by pumping a fluoride-glass fiber with a picosecond mode-locked Tm-doped fiber laser. The spectral power density was measured to be 1 mW/nm. The laser was coupled to a Thermo Scientific 8700 FTIR bench and a Continuum IR microscope to image a human liver sample by mapping the absorption bands in the "CH" region. The spectral maps reveal presence of lipid and provide qualitative and quantitative information about the chemical content. Our high-power laser provides high quality images with eightfold decrease in acquisition time compared to FTIR internal thermal source when using 10x10 μm2 aperture. Then, we recorded high resolution maps using a 3x3 μm2 aperture. We obtained ~1% rms noise level with the laser source. This performance that could not be achieved with the thermal source due to its low brightness is similar to that obtained with the synchrotron source.

10060-9, Session 3

Taking label-free optical spectroscopy techniques into the operating theatre: biopsy needles and surgical guidance probes *(Invited Paper)*

Frédéric Leblond, Ecole Polytechnique de Montréal (Canada)

Recent advances will be described relating to the development and clinical translation of optical spectroscopy techniques designed to guide surgical interventions in brain and prostate oncology applications. The use of molecular imaging guidance systems can enable true intra-operative tissue identification, increasing the effectiveness of cancer surgery and potentially positively impacting patient survival.

Surgical resection is a fundamental cancer treatment, but its effectiveness is reduced by the inability to rapidly and accurately identify cancer margins. We will introduce a portable intraoperative label-free multimodal optical spectroscopy system combining intrinsic fluorescence, diffuse reflectance, and Raman spectroscopy that can identify cancer in situ during surgery. We will show that this on-line guidance system can detect primary cancer such as glioma as well as metastatic melanoma and cancer of the lung and colon with an accuracy, sensitivity, and specificity of 97%, 100%, and 93% respectively. Moreover, a method will be presented, along with preliminary tissue classification results, based on the interrogation of whole human prostates from prostatectomies.

The development and in vivo validation of an optical brain needle biopsy instrument will be presented demonstrating its ability to detect bulk tumor using Raman spectroscopy with the goal of reducing the number of non-diagnostic samples during a procedure. The extraction of tissue can cause life-threatening hemorrhage because of significant blood vessel injury during the procedure. We will demonstrate that a sub-diffuse optical tomography technique integrated with a commercial biopsy needle can detect the presence of blood vessels to limit the hemorrhage risk.

10060-10, Session 3

Intra-opeartive OCT imaging and sensing devices for clinical translation *(Invited Paper)*

Yu Chen, Univ. of Maryland, College Park (United States)

Stereotactic procedures that require insertion of needle-based instruments into the brain serve important roles in a variety of neurosurgical interventions, such as biopsy, catheterization, and electrode placement. A fundamental limitation of these stereotactic procedures is that they are blind procedures in that the operator does not have real-time feedback as to what lies immediately ahead of the advancing needle. Therefore, there is a great clinical need to navigate the instrument safely and accurately to the targets. Towards that end, we developed a forwarding-imaging needle-type optical coherence tomography (OCT) probe for avoiding the hemorrhage and guiding neurosurgical interventions. The needle probe has a thin diameter of 0.7 mm. The feasibility of vessel detection and neurosurgical guidance were demonstrated on sheep brain in vivo and human brain ex vivo. In addition, we further reduced the probe size to 0.3 mm using an optical Doppler sensing (ODS) fiber probe that can integrate with microelectrode recording (MER) to detect the blood vessels lying ahead to improve the safety of this procedure. Furthermore, to overcome the field-of-view limitation of OCT probe, we developed an MRI-compatible OCT imaging probe for

neurosurgery. MRI/OCT multi-scale imaging integrates micro-resolution optical imaging with wide-field MRI imaging, and has potential to further improve the targeting accuracy.

10060-11, Session 3

Real-time assessment of breast surgical margins with fluorescence-guided OCT imaging *(Invited Paper)*

Nicusor V. Iftimia, Jesung Park, Gopi N. Maguluri, Physical Sciences Inc. (United States); Savitri Krishnamurthy, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States)

A novel multimodal optical imaging approach for real-time assessment of surgical margins on breast cancer lumpectomy specimens is presented. Our approach is to target cancer cells using an optically silent peptide substrate containing two (NIR) fluorochromes, internally quenched, which are cleaved by highly expressed breast cancer enzymes, like urokinase-type plasminogen activator (uPA). Thus this agent becomes highly fluorescent only on the cancer area when the specimen is excited by a NIR laser beam. A fluorescence imager is used to highlight cancer-suspect margins on the surgical specimen, while high-resolution optical coherence tomography (OCT) imaging is used to visualize tissue morphology on the highlighted areas and confirm or rule out cancer presence. This technology will hopefully increase the success rate of cancer surgeries, reduce the risk of cancer recurrence and significantly reduce US healthcare costs.

10060-12, Session 3

Confocal laser endomicroscopy for brain tumor surgery: a milestone journey from microscopy to cellular surgery *(Invited Paper)*

Cleopatra Charalampaki, Medical Ctr. Cologne (Germany)

The aim in brain tumor surgery is maximal tumor resection with minimal damage of normal neuronal tissue. Today diagnosis of tumor and definition of tumor borders intraoperatively is based on various visualization methods as well as on the histopathologic examination of a limited number of biopsy specimens via frozen sections. Unfortunately, intraoperative histopathology bears several shortcomings, and many biopsies are inconclusive. Therefore, the desirable treatment could be to have the ability to identify intraoperative cellular structures, and differentiate tumor from normal functional brain tissue on a cellular level. To achieve this goal new technological equipment integrated with new surgical concepts is needed.Confocal Laser Endomicroscopy (CLE) is an imaging technique which provides microscopic information of tissue in real-time. We are able to use these technique to perform intraoperative “optical biopsies” in bringing the microscope inside to the patients brain through miniaturized fiber-optic probes, and allow real-time histopathology.

In our knowledge we are worldwide the only one neurosurgical group using CLE intraoperative for brain tumor surgery. We can detect and characterize intraoperative tumor cells, providing immediate online diagnosis without the need for frozen sections. It also provides delineation of borders between tumor and normal tissue on a cellular level, making surgical margins more accurate than ever before. The applications of CLE-assisted neurosurgery help to accurate the therapy by extending the resection borders and protecting the functionality of normal brain tissue in critical eloquent areas.

10060-13, Session 4

Simultaneous white-light and protoporphyrin-IX fluorescence imaging for optimized cystoscopic detection of non-muscle-invasive bladder cancer

Stavros G. Demos, Ronald W. Wood, Univ. of Rochester (United States)

Detection of non-muscle-invasive bladder carcinoma (especially of flat lesions), in situ and low-grade tumors remains a challenge. Recent studies have shown that the detection of non-muscle-invasive bladder carcinoma lesions is enhanced by utilizing blue-light fluorescence cystoscopy after instilling a photosensitizing drug such as 5 aminolevulinic acid or hexyl-aminolevulinate. Cysview™ is the only FDA-approved imaging agent for this purpose.

Exciting the tissue with blue light produces red emission by protoporphyrin IX that results from uptake and transformation of the contrast agent. Currently, an initial white-light survey of the bladder is followed by a survey under the blue-light imaging mode; this is performed with a single camera and high-intensity light source with a filter wheel. Blue-light cystoscopy has been reported to improve tumor detection and resection, thereby reducing the likelihood of disease recurrence. However, the use of two imaging modes in alternation prolongs a clinical procedure already lengthened by the current requirement of a 1-h pretreatment with a contrast agent.

We have developed a prototype multispectral endoscope to optimize sensitivity and image quality for both reflectance and fluorescence imaging modalities. Because the images are captured concurrently, there is no need to switch between imaging modes. The surgeon is free to alternate between them, observe both simultaneously, or can rely on digital-image–processing methods to merge them into a single-image stream. The imaging instrument interfaces with an ordinary cystoscope or endoscope and promises to enhance endoscopic imaging in multiple contexts.

10060-14, Session 4

Hybrid Raman spectroscopy and optical coherence tomography technique for the diagnosis of oral malignant lesions

Jianfeng Wang, Wei Zheng, Kan Lin, Zhiwei Huang, National Univ. of Singapore (Singapore)

In this work, hybrid Raman spectroscopy (RS) and optical coherence tomography (OCT) technique was employed for the oral malignant lesions diagnosis. A side-view handheld RS-OCT optical probe is designed to co-align the optical paths of RS and OCT sampling arms. While RS reveals Raman spectral differences between normal and malignant oral tissues that can be attributed to the differences in inter- and intra-cellular proteins, lipids, DNA and water structures and conformations, enlightening biochemical changes associated with oral malignancy development; Simultaneously, OCT casts light on the tissue morphology changes accompanying the oral malignancy.

10060-16, Session 4

Mechanism and applications of new fluorescent compounds produced by femtosecond laser surgery in biological tissue *(Invited Paper)*

Jianan Y. Qu, Qiqi Sun, Hong Kong Univ. of Science and Technology (Hong Kong, China)

The single or multi-photon microscopy based on fluorescent labelling and staining is a sensitive and quantitative method that is widely used in molecular biology and medical research for a variety of experimental, analytical, and quality control applications. However, label-free method is highly desirable in biology and medicine when performing long term live imaging of biological system and obtaining instant tissue examination during surgery procedures. Recently, our group found that femtosecond laser surgery turned a variety of biological tissues and protein samples into highly fluorescent substances. The newly formed fluorescent compounds produced during the laser surgery can be excited via single- and two-photon processes over broad wavelength ranges. We developed a combined confocal and two-photon spectroscopic microscope to characterize the fluorescence from the new compound systematically. The structures of the femtosecond laser treated tissue were studied using Raman spectroscopy and transmission electron microscopy. Our study revealed the mechanisms of the fluorescence emission form the new compound. Furthermore, we demonstrated the applications of the fluorescent compounds for instant evaluation of femtosecond laser microsurgery, study of stem cell responses to muscle injury and neuro-regeneration after spinal cord injury.

10060-17, Session 4

Two-photon microscopy optical biopsy probe using a fiber-based supercontinuum source *(Invited Paper)*

Youbo Zhao, Physical Science Inc. (United States); Gopi N. Maguluri, Jesung Park, Physical Sciences Inc. (United States); Nicusor V. Iftimia, Physical Science Inc. (United States)

Two-photon fluorescence microscopy (2PM) holds a great promise for noninvasive histopathology or optical biopsy. Despite its well-known advantages, the clinical translations of 2PM have been substantially hampered, largely because of several technological and cost barriers. The cost of a 2PM system is often exceedingly high (e.g., >$500k), which is primarily driven by the required femtosecond laser. Moreover, it is still a big challenge to deliver the femtosecond pulses from the bulky laser sources to the tissue sites (pathological targets) through a flexible optical channel, such as an optical fiber. Especially for ultra-broadband or wavelength tunable femtosecond pulses, which are needed to efficiently excite different fluorophores, the temporal distortion induced by the chromatic dispersion in an optical fiber can be severe and hard to mitigate.

Here we present a low-cost approach to generate fiber-based/fiber-delivered broadband femtosecond pulses for ultra-high resolution multiphoton imaging. This system consists of a dispersion-compensated laser-delivery fiber and a supercontinuum generation fiber. The laser-delivery fiber module is composed of a glass rod and a hollow-core (HC) fiber which delivers high-power distortion-free femtosecond pulses (up to 500 mW of ~100 fs pulses centered at 800 nm). These fiber-delivered, relatively narrowband laser pulses are then coupled into a short piece of high-nonlinearity photonic crystal fiber to generate a broadband supercontinuum (730 nm – 870 nm). This femtosecond supercontinuum source, generated at the distal end of the fiber module, is well-suited for handheld or endoscopic 2PM probes, which might be suitable for noninvasive optical biopsy.

10060-18, Session 4

Hyperspectral imaging based on compressive sensing to determine cancer margins in human pancreatic tissue ex vivo

Joseph A. Peller, The Univ. of North Carolina at Charlotte (United States); Kyle J. Thompson, Imran Siddiqui, John Martinie, David A. Iannitti, Carolinas Medical Ctr. (United States); Susan R. Trammell, The Univ. of North Carolina at Charlotte (United States)

Pancreatic cancer is the fourth leading cause of cancer death in the United States. Most pancreatic cancer patients will die within the first year of diagnosis, and just 6% survive five years. Currently, surgery is the only treatment that offers a chance of cure for pancreatic cancer. However, accurately identifying tumor margins in real-time is a significant difficulty during surgery and contributes to the low 5-year survival rate. We are developing a single-pixel hyperspectral imaging system based on compressive sensing for real-time tumor margin detection to facilitate more effective removal of diseased tissue. Our spectral imaging system uses autofluorescencent emission from collagen (400 nm) and NAD(P)H (475 nm) and reflectance spectroscopy as diagnostics for differentiating between healthy and diseased tissue. In this study, we demonstrate the ability of our imaging system to discriminate between healthy pancreatic tissue and adenocarcinoma (AC). Ten ex vivo human pancreatic tissue samples were imaged immediately after excision using our hyperspectral system. Both the autofluorescence and reflectance spectra differed between AC and healthy tissue. Multiple 2-D spectral images were analyzed using spectral angle mapping to highlight differences between the tissue types. Margins of the diseased regions were determined based on differences in the spectral angle across the image in both fluorescence and reflectance images. Margins determined via imaging were in good agreement with margins seen via histology.

10060-19, Session 4

In vivo analyzing of bone mineral density with near infrared light

Chung Chun, National Chiao Tung Univ. (Taiwan)

Osteoporosis is a common disease in an aging society. Although it is a major influence on health of the aged, it is not deadly. Therefore, people do not pay attention on it until condition deteriorated. For this reason, the prior diagnosis becomes important.

Presently major measuring methods of bone mineral density(BMD) are dual-energy x-ray absorptiometry(DXA) and ultrasound bone densitometer(USBD).The advance of DXA is high accuracy. However, it has the risk of radiation exposure and it takes long time. For USBD, the process is simple and fast, but the accuracy is not exact. So we develop a novel method to measure BMD with near infrared (NIR). The purposes of our system are to measure BMD simply and to get precise data.

In our experiment, we set up a transmissive system that emits NIR into patients' wrist and obtains image, and analyze the data and compare the data with DXA data which patients have done before our test. We found a good correlation between measured data of our system and DXA.

10060-20, Session 4

Study of anti cancer effects of chemotherapeutic agents and radiotherapy in breast cancer patients using fluorescence spectroscopy

Chithra Krishnamoorthy, Aruna Prakasa Rao, Anna Univ., Chennai (India); Vijayaraghavan Srinivasan, Paterson Cancer Ctr. (India); Ganesan Singaravelu, Anna Univ., Chennai (India)

The analysis of the variations in the spectroscopic patterns of the key bio molecules using Native fluorescence spectroscopy, without exogenous labels has emerged as an intrinsic parameter in the characterization of the Physiological State and the Discrimination of Pathological from normal conditions of cells and tissues as the relative concentration of these bio-molecules serves as a marker in evaluating the presence of cancer in some

organ or tissue of the body. The aim of this unique study was to use these features of Optical spectroscopy in monitoring the behavior of cells to treatment and thus to evaluate the treatment response to Chemotherapeutic agents and Radiation in Breast Cancer Patients. The results are promising, enhancing the scope of Native fluorescence Spectroscopy emerging as a promising tool in the Evaluation of Therapeutic Response in Cancer Patients.

10060-43, Session PTues

Characterization of urine and semen samples of normal and prostate cancer by FTIR spectroscopy

Ramu Rajasekaran, Alexandre Carneiro Braga, Thiago de Oliveira Mendes, Airton Abrahao Martin D.D.S., Univ. do Vale do Paraíba (Brazil)

World Health Organization (WHO) reported that,cancer is one among the leading causes of morbidity and mortality and areexpected torise about 13 million by the year 2030. The incidence of cancer is increasing worldwide due to change in life style such as tobacco usage, alcohol consumption, obesity, physical inactivity etc. In Brazil, Prostate cancer is the most common cancer among the men and responsible for increased cancer deaths.Most prostate cancers are first found during screening with a prostate-specific antigen (PSA) blood test, biopsy or a digital rectal exam (DRE).Early prostate cancers usually do not cause symptoms, but more advanced cancers are sometimes first found because of symptoms they cause resulting in poor survival and high mortality rates. Though biopsy is considered as a reliable, direct method and gold standard in the clinical diagnosis of cancer, it is invasive and painful. All these bring complication and artifacts. Hence, our aim is to search a simple, non-invasive and inexpensive protocol for the diagnosis of cancer in its early stage. Recent reports showed the possibility of urine spectroscopy in the diagnosis of cancer. In this regard, we aimed at FTIR spectroscopic characterizing the bio fluids which are not invasive in collection such as urine and semen samples of normal and prostate cancer. The results of the spectral variations and the correlation of results of urine and semen samples will be discussed.

10060-44, Session PTues

Resonance Raman of BCC and normal skin sliced samples with depth dependence

Cheng-Hui Liu, Vidyasagar Sriramoju, The City College of New York (United States); Susie Boydston-White, Borough of Manhattan Community College (United States); Binlin Wu, Southern Connecticut State Univ. (United States); Chunyuan Zhang, Zhe Pei, Laura A. Sordillo, The City College of New York (United States); Hugh Beckman M.D., Sinai Eye Education Corp. (United States); Robert R. Alfano, The City College of New York (United States)

The Resonance Raman (RR) spectroscopy has been demonstrated as a practical approach for minimally invasive and early detection of human diseases. This study is to evaluate the effects of biomarkers to distinguish basal cell carcinoma (BCC) at different depths from normal human skin tissues.

A WITec R300 Confocal micro Raman spectrometer was used to measure the biochemical changes as function of the BCC skin depth. The RR spectra were measured from BCC and normal sliced skin samples, including 28 vertical section slices of BCC samples and 8 horizontal section slices of normal samples. Each slice is 50μm thick, approximately 8400μm long and 5600μm wide. One vertical section slice of BCC sample was measured at 6 depths with step 100μm separation, and 9 depths with step 500um separation starting at the top site of surface. In total 54 spectra were analyzed.

The results of the comparisons of the changes in RR biomarkers showed that in the first layer of 100 μm below the surface which is in the epidermis and dermis region had a biggest enhancement in proteins including collagen combined amide I and amino acids, and dramatically decreased in carotenoids and lipid and lipid protein contents.

The diagnostic algorithms for the classification of BCC and normal were developed based on SVM classifier and PCA statistical method. These statistical methods were used to analyze the RR spectral data collected from skin tissues, yielding a diagnostic sensitivity of 98.7% and specificity of 79% compared with histopathology reports.

10060-45, Session PTues

Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) in the discrimination of normal and oral cancer blood plasma

Rekha Pachaiappan, Aruna Prakasa Rao, Ganesan Singaravelu, Anna Univ., Chennai (India)

Oral cancer is the most frequent type of cancer that occurs with 75000 to 80000 new cases reported every year in India. The carcinogens from tobacco and related products are the main cause for the oral cancer. ATR-FTIR method is label free, fast and cost-effective diagnostic method would allow for rapid diagnostic results in earlier stages by the minimal chemical changes occur in the biological metabolites available in the blood plasma. The present study reports the use of ATR-FTIR data with advanced statistical model (LDA-ANN) in the diagnosis of oral cancer from normal with better accuracy. The infrared spectra were acquired on ATR-FTIR Jasco spectrophotometer at 4 cm-1 resolution, 30 scans, in the 1800-900 cm-1 spectral range. Each sample had 5 spectra recorded from each blood plasma sample. The spectral data were routed through the multilayer perception of artificial neural network to evaluate for the statistical efficacy. Among the spectral data it was found that amide II (1486 cm-1) and lipid (1526 cm-1) affords about 90 % in the discrimination between groups using LDA. These preliminary results indicate that ATR-FTIR is useful to differentiate normal subject from oral cancer patients using blood plasma.

10060-46, Session PTues

Oral cancer detection based on fluorescence polarization of blood plasma at excitation wavelength 405 nm

Rekha Pachaiappan, Aruna Prakasa Rao, Yuvaraj Manoharan, Anna Univ., Chennai (India); Koteeswaran Dornadula, Meenakshi Ammal Dental College & Hospital (India); Ganesan Singaravelu, Anna Univ., Chennai (India)

During metabolism the metabolites such as hormones, proteins and enzymes were released in to the blood stream by the cells. These metabolites reflect any change that occurs due to any disturbances in normal metabolic function of the human system. This was well observed with the altered spectral signatures observed with fluorescence spectroscopic technique. Previously many have reported on the significance of native fluorescence spectroscopic method in the diagnosis of cancer. As fluorescence spectroscopy is sensitive and simple, it has complementary techniques such as excitation-emission matrix, synchronous and polarization. The fluorescence polarization measurement provides details about any association or binding reactions and denaturing effects that occurs due to change in the micro environment of cells and tissues. In this study, we have made an attempt in the diagnosis of oral cancer at 405 nm excitation using fluorescence polarization measurement. The fluorescence anisotropic values calculated from polarized fluorescence spectral data of normal and oral cancer subjects yielded a good accuracy when analyzed with linear discriminant analysis based artificial neural network. The results will be discussed in detail.

10060-47, Session PTues

Characterization and classification of oral tissues using excitation and emission matrix: a statistical modeling approach

Udayakumar Kanniyappan, Univ. of Maryland, College Park (United States); Aruna Prakasa Rao, Anna Univ., Chennai (India); Koteeswaran Dornadula, Meenakshi Ammal Dental College & Hospital (India); Ganesan Singaravelu, Anna Univ., Chennai (India)

Oral cancer is considered as the dominant threatening disease among other types of cancer, especially in asian countries. Though the rapid technology in optics and engineering has grown tremendously in the past few years, a simple diagnostic modality for early detection of oral cancer has not been emerged successfully. For the past few decades, much interest has been developed towards optical spectroscopy based diagnosis for various types of diseases including cancer. Among them, fluorescence spectroscopy has been successfully considered as the rapid diagnostic tool in oncological applications. Native fluorescence spectroscopy in detection of precancerous lesion has been considered for long years, but the present study explores the Parallel Factor Analysis (PARAFAC) as diagnostic tool to discrimination malignant, premalignant from normal tissues. Parallel Factor Analysis (PARAFAC) is the statistical modelling approach which isolates the finger print of the endogenous fluorophores present in tissues by mathematically and chemically using Excitation Emission Matrix (EEM), in which it provides the wealth of information about the altered tissue metabolism. Each key factor is identified with their excitation and emission spectra along with the fluorescent intensity scores. Then the scores were subjected to statistical analysis (Student's t test and Linear Discriminant Analysis) to check the diagnostic potentiality of the present technique. Hence, the present study may explore the application of Excitation Emission Matrix (EEM) in oral cancer diagnosis using PARAFAC analysis to explore the possibility of alternate tool in oral cancer diagnosis.

10060-48, Session PTues

Quantification of hemoglobin and its derivatives in oral cancer diagnosis by diffuse reflectance spectroscopy

Udayakumar Kaniyappan, Univ. of Maryland, College Park (United States); Einstein Gnanatheepam, Anna University (India); Aruna Prakasa Rao, Anna Univ., Chennai (India); Koteeswaran Dornadula, Meenakshi Ammal Dental College & Hospital (India); Ganesan Singaravelu, Anna Univ., Chennai (India)

Every year cancer death rate increases at an alarming rate in developing countries as well as around the world. At global stage, India ranks first in highest number of oral cancer incidence. Among various optical spectroscopic techniques, diffuse reflectance spectroscopy (DRS) has been established as one of the simplest, cost effective and rapid techniques in the classification of tissues and to understand the structural and morphological changes in tissues under different pathophysiological conditions. Estimation of hemoglobin concentration plays an important role in diseases like anemia, angiogenesis and it includes different types of cancer (breast, oral, and colon, etc.). Hence, it warrants a detailed study on quantification of hemoglobin concentration of normal and malignant oral tissues is necessary to probe the altered hemoglobin metabolism. To measure tissue reflectance, a home-made sample holder designed indigenously and was successfully calibrated with the commercially available integrating sphere accessories (RSA-PE-20 integrating sphere accessory, Perkin-Elmer Lambda 35) using UV-Vis spectrophotometer. The averaged diffuse reflectance spectra of forty normal and malignant oral tissues confirm the presence of various biomarkers including beta-carotene and methemoglobin. To study the statistical significance of the averaged diffuse reflectance spectra of normal and malignant oral tissues, the stepwise LDA was performed. Further, the oxy hemoglobin (HbO2), deoxy hemoglobin (Hb), total hemoglobin (tHb) concentrations and hemoglobin oxygen saturation (StO2) were estimated using diffuse reflectance spectra of normal and malignant oral tissues. The statistical significance of the quantified hemoglobin and its derivatives is validated to know whether this technique has any diagnostic potential in discriminating malignant from normal tissues.

10060-49, Session PTues

Supercontinuum imaging of diseases of the bone and joints

Laura A. Sordillo, The City College of New York (United States); Diana C. Sordillo, The City Univ. of New York (United States); Peter P. Sordillo M.D., The City College of New York (United States) and Lenox Hill Hospital (United States); Lingyan Shi, The City College of New York (United States) and Columbia Univ. (United States); Robert R. Alfano, The City College of New York (United States)

The conventional near-infrared (NIR) therapeutic window, located at wavelengths between 650 and 950 nm, has been utilized for numerous biomedical applications. At longer NIR wavelengths, Rayleigh scattering (varying as 1/?4) and Mie scattering (at 1/?n for n≥1) are minimized, thus allowing for higher quality images. Located between water peak maxima, there exist additional NIR optical windows, a second (located between 1100 to 1350 nm), third (from 1600 to 1870 nm) and fourth NIR window (from 2,100 to 2,350 nm), beyond the first NIR optical window. We previously studied the optical properties of normal and diseased tissues at longer NIR wavelengths, and found that the total attenuation lengths were longest at longer NIR wavelengths (most notably at the third window). Since that time, the optical properties of tissues obtained from rat brain were analyzed, and it was determined that the third NIR optical window is also the ideal window for imaging of the brain. We report the use of an intense supercontinuum (SC) laser light system for the assessment of benign and malignant abnormalities of the bone and joints. Advantages of the supercontinuum laser light system include high power, broadband spectrum (can span from the UV to the infrared (IR)), and the ability to use it in combination with many optical applications such as fluorescence lifetime imaging and Förster resonance energy transfer (FRET). Data on osteoporosis, osteogenesis imperfecta, Paget's disease of bone, osteoarthritis and osteomalacia, as well as on benign and malignant tumors and tumor metastases to bone, will be presented.

10060-50, Session PTues

NIR ballistic supercontinuum laser system for the evaluation of non-small cell lung carcinoma, malignant carcinoid, breast carcinoma and other cancers

Laura A. Sordillo, The City College of New York (United States); Lingyan Shi, The City College of New York (United States) and Columbia Univ. (United States); Peter Sordillo, The City College of New York (United States) and Lenox Hill Hospital (United States); Robert R. Alfano, The City College of New York (United States)

The second (1100-1350 nm) and third NIR windows (1600-1870 nm) have been utilized with label-free linear and multiphoton imaging to study the brain and breast, and the use of a fourth window (2100-2350 nm) is now being explored. Located in regions of minimal scattering, these NIR windows allow increased depth penetration through tissue and may thus provide high contrast images. We report on the use of a ballistic supercontinuum laser

system to assess malignant and normal tissues from patients with cancers including non-small cell lung carcinoma, malignant carcinoid and breast carcinoma. Optical attenuation measurements from these tissue samples were obtained.

10060-55, Session PTues

Synchrotron micro-CT imaging reveals cranial nerves and vessels in juvenile zebrafish

Xuying Xin, Keith C. Cheng, Jake Gittlen, Penn State Hershey (United States)

Comprehensive measurement of morphological phenotypes caused by gene function and environment optimally includes detection of changes in 3D tissue architecture in the context of the whole organism, but is challenged by limitations of resolution, field of view and tissue opacity. The accumulation of pigment associated with organogenesis causes tissue opacity that degrades imaging that uses visible light. We describe the potential of x-ray based, micron resolution whole-organism tissue tomography to overcome these barriers. To illustrate the utility of Synchrotron micro CT in the study of morphological phenotyping, we have identified major cranial nerves and blood vessels of individual juvenile zebrafish.

10060-21, Session 5

Resonance Raman scattering of ?-carotene using second singlet state *(Invited Paper)*

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Resonance Raman (RR) is becoming important in biomedical application for native chromophores such as carotenoids. Carotene is one of most widespread molecules in plants and animals and is involved in various processes in the human body . In our study, the Raman spectra of ?-carotene solutions were excited by 488 nm, 514 nm, 532 nm and 633 nm of visible laser beams, which exhibited significant RR enhancement and obvious fluorescence when the laser energy became closer to electronic transition energy from S0 to Sj for j=2. Using the method of self-absorption correction, we could obtain original Raman intensity and actual resonance Raman gain without self-absorption from S2 by ?-carotene molecules, and thus evaluated the effect of self-absorption on RR scattering. Although the wavelengths of 488 nm and 514 nm seemed better for stronger RR enhancement, our results indicated that 532 nm should be optimum Raman pump laser with moderate resonance Raman enhancement at reduced fluorescence and least affected by self-absorption. The 532 nm excitation will be helpful for making use of resonance Raman spectroscopy to investigate biological molecules and tissues.

10060-22, Session 5

Raman spectroscopy for cancer detection and characterization in metastasis models *(Invited Paper)*

Shigehiro Koga M.D., Yusuke Oshima, Mitsunori Sato, Kei Ishimaru M.D., Motohira Yoshida M.D., Yuji Yamamoto M.D., Yusuke Matsuno M.D., Yuji Watanabe M.D., Ehime Univ. (Japan)

Raman spectroscopy provides a wealth of diagnostic information to the surgeon with in situ cancer detection and label-free histopathology in clinical practice. Raman spectroscopy is a developing optical technique which can analyze biological tissues with light scattering. The difference in frequencies between the incident light and the scattering light are called Raman shifts, which correspond to the vibrational energy of the molecular bonds. Raman spectrum gives information about the molecular structure and composition in biological specimens. We had been previously reported that Raman spectroscopy could distinguish various histological types of human lung cancer cells from normal cells in vitro. However, to identify and detect cancer diagnostic biomarkers in vivo on Raman spectroscopy is still challenging, because malignancy can be characterized not only by the cancer cells but also by the environmental factors including immune cells, stroma cells, secretion vesicles and extracellular matrix. Here we investigate morphological and molecular dynamics in both cancer cells and their environment in xenograft models and spontaneous metastasis models using Raman spectroscopy combined with fluorescence microscopy and photoluminescence imaging. We are also constructing a custom-designed Raman spectral imaging system for both in vitro and in vivo assay of tumor tissues to reveal the metastasis process and to evaluate therapeutic effects of anti-cancer drugs and their drug delivery toward the clinical application of the technique.

10060-23, Session 5

An interactive visual interface for the determination of similarity patterns in the Fourier spatial frequency spectrum of laser speckle

Stewart Russell, Sam Payne, Lisa Chan, The City College of New York (United States)

Laser speckle scattered by particles in solution smaller than the wavelength of the applied light resemble a random Gaussian field. However, the time varying signal does contain a characteristic pattern when analyzed in frequency space. We have previously shown that the time correlated rate of change in total variance is related to the diffusivity of the nanoscale particles. The basis Airy disk is formed by the instantaneous microstructure of these particles, and a time dependent fluctuation in the intensity of characteristic Fourier spatial frequencies is determined by the rate at which the microstructure reorganizes to form new scattering surfaces. In this paper we demonstrate an interactive tool to allow for the investigation of characteristic frequencies to detect similarities and differences between such seemingly random scattering fields. We reconstruct an idealized image from the noise-reduced Fourier spatial frequency power spectrum (FSPS), by taking the inverse transform of a reduced set of the full spectrum. We extract the FSPS from laser speckle from nanoparticles in suspension, and optimize the spectrum using our recently developed methods. When the optimized Fourier spatial frequency spectrum is inverse transformed, a representative pattern emerges. When applied to time-resolved laser speckle, this lattice average gives a representation of how fast the intensity map fluctuates and can be directly correlated to the transport properties of the particles.

10060-24, Session 5

Continuous monitoring of cerebral hemodynamics during extracorporeal membrane oxygenation therapy

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Kaitlin R. Griffith, Peter J. Schwab, The Children's Hospital of Philadelphia (United States); Erin M. Buckley, Georgia Institute of Technology (United States); Todd J. Kilbaugh, The Children's Hospital of Philadelphia (United States); Arjun G. Yodh, Univ. of Pennsylvania (United States); Daniel J. Licht, The Children's Hospital of Philadelphia (United States)

Extra corporal membrane oxygenation (ECMO) is the ultimate, temporary treatment modality for >1500 children each year with life-threatening cardiopulmonary disorders. During ECMO, the lungs and/or heart are bypassed through large-vessel cannulation to permit mechanical blood oxygenation and perfusion. Devastating neurologic injury is a frequent complication, potentially due to profoundly deranged neurovascular physiology. Cerebral Auto-Regulation (CAR) is an intrinsic mechanism to maintain appropriate perfusion and oxygenation despite systemic variations. Maintaining normal CAR on ECMO may be critical for patients. However, ECMO pump-rates are determined by anthropomorphic values and slowly-varying clinical observations. Understanding the relationship of CAR with empiric ECMO flows in-vivo may help develop goal-directed protocols for ECMO care.

Diffuse optical and correlation spectroscopies provide rapid, quantitative, and non-invasive measurements of tissue blood oxygenation, volume, and blood flow, as validated against a number of clinical techniques and animal models. We have applied these tools to pediatric ECMO patients during manipulation of the ECMO pump rate.

We assessed cerebral blood flow during adjustments of ECMO parameters using diffuse optical and correlation spectroscopies in 17 measurements of 10 subjects. We observe both regulated (constant) and passive (pressure dependent) cerebral blood flows during ECMO flows titrations.

Diffuse optical spectroscopies provide first time evidence of dysregulated cerebral perfusion during ECMO therapy: we observe both regulated and passive, pressure-dependent, flow. Correlation with long term outcome is warranted. Better understanding of cerebral perfusion of ECMO patients could eventually lead to individualized titration of ECMO flow settings and reduce the risk of neurologic injury.

10060-25, Session 5

Lesion transmurality assessment using multi-fiber diffuse reflectance

Rajinder P. Singh-Moon, Christine P. Hendon, Columbia Univ. (United States)

In non-pharmacological treatment of cardiac arrhythmias such as catheter ablation therapy, long-term treatment effectiveness is related in part to the quality of lesion generation. Superficial lesions may lead to arrhythmia recurrence by allowing recovery along conduction channels for arrhythmic impulses to propagate; conversely transmural lesions inhibit conduction. Conventional techniques rely on measurement of surrogate parameters such as change in bioelectrical impedance, or electrogram amplitude dampening as a qualitative assessment for lesion size. In previous work, we've demonstrated a relationship between lesion dimensions and spectroscopic parameters extracted using an optically-integrated ablation catheter. Though these metrics present some trend, a method to directly assess lesion transmurality maybe better suited. In this work, we report a method for direct recovery of lesion depth in cardiac tissue using diffusely reflected optical measurements and present initial in silico validation.

Photon transport throughout a heterogeneous volume was simulated for a series of source-detector pairs and optical properties using a GPU-based Monte Carlo (MC) code. Results were used to generate a multi-dimensional look-up table for each collection geometry for partial to transmural lesions. A genetic algorithm-based two-step inversion method was employed to extract lesion transmurality. MC simulated optical measurements for various lesion sizes were generated using optical properties for ablated and normal cardiac tissue found in literature and were fitted using our algorithm. Recovered lesion depths ranged between 2-10% for lesions less than 3mm and were within 20% for lesions greater than 4mm. These results support the application of this technique for lesion validation for atrial tissue.

10060-26, Session 5

Depth-sensitive optical spectroscopy for layered tissue measurements

Wei Liu, Xiaojun Yu, Quan Liu, Linbo Liu, Yi Hong Ong, Nanyang Technological Univ. (Singapore)

Disease diagnosis based on the visual inspection of the pathological presentations or symptoms on the epithelial tissue such as the skin are subjective and highly depend on the experience of the doctors. Vital diagnostic information for the accurate identification of diseases is usually located underneath the surface and its depth distribution is known to be related to disease progression. Although optical spectroscopic measurements are fast and non-invasive, the accurate retrieval of the depth-specific diagnostic information is complicated by the heterogeneous nature of epithelial tissues. The optical signal measured from a tissue is often the result of averaging from a large tissue volume that mixes information from the region of interest and the surrounding tissue region, especially from the overlaying layers. Our group has developed a series of techniques for depth sensitive optical measurements from such layered tissues. We will first review the earlier development of composite fiber-optic probe, in which the source-detector separation and the angles of source and detector fibers are varied to achieve depth sensitive measurements. Then the more recent development of non-contact axicon lens based probes for depth sensitive fluorescence measurements and the corresponding numerical methods for optimization will be introduced. Finally, the most recently developed snapshot axicon lens based probe that can measure Raman spectra from five different depths at the same time will be discussed. Results from tissue phantoms, ex vivo pork samples and in vivo fingernail measurements will be presented, which indicates the great potential of depth sensitive optical spectroscopy for clinical tissue diagnosis.

10060-15, Session 6

Fiber sensor assisted in-vivo needle guidance for minimally invasive procedures

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Image-guided procedures are performed frequently by radiologists to insert a catheter within a target vessel or lumen or to perform biopsy of a lesion. For instance, an interventional radiologist uses fluoroscopy during percutaneous biliary drainage procedure (a procedure during which a catheter is inserted through the skin to drain the bile from liver) to identify the location of the needle tip within liver parenchyma, hepatic blood vessel or bile duct.

However, the identification of the target organ under fluoroscopy exposes the patient to x-ray irradiation, which may be significant if the time of procedure is prolonged.

We have designed a fiber core needle system that may help the radiologist identify the location of the needle tip in real time without exposing the patient to x-ray. Our needle system transmits a low power modulated light into the tissue through a fiber cable embedded in the needle and detects the backscattered light using another fiber inside the needle. We were able to successfully distinguish the location of our prototype needle tip inside a cow liver phantom to identify if the needle tip was within liver parenchyma, liver vessels, or in the bile duct based on the recorded backscattered light.

10060-27, Session 6

Nonlocal correlations of polarization-entangled photons through brain tissue *(Invited Paper)*

Enrique J. Galvez, Colgate Univ. (United States); Lingyan Shi, Columbia Univ. (United States); Robert R. Alfano, The City College of New York (United States)

We investigated the preservation of non-local correlations between polarization-entangled photons when one of them traveled through brain tissue slices of different thicknesses. Using down-converted photons at a wavelength of 802 nm minimized the absorption by the tissue. After the light passed through the tissue samples, we performed quantum state tomography to obtain quantitative measures of the entanglement. We found that entanglement is preserved to a surprising degree, and when it degrades, it does so following a particular path in a tangle versus linear-entropy graph. Such a trajectory reveals direct transfer of probability from entangled to mixed state.

10060-28, Session 6

Automated optical biopsy with the circularly polarized light *(Invited Paper)*

Alexander Bykov, Sami Huttunen, Univ. of Oulu (Finland); Alexander Doronin, Yale Univ. (United States); Alexey P. Popov, Markus Mäkinen, Igor Meglinski, Univ. of Oulu (Finland)

In this paper, we report on the development of automated system for optical diagnostics of biotissues with circularly polarized light. In the setup, the right circularly polarized light is focused onto the sample with a lens. The probing beam (640 nm, 30 mW) is tilted from the sample normal at 55°. The backscattered light is collected at a distance of 0.5 mm from the point of incidence. The polarization state of the detected light is then analyzed with the polarimeter. The source-detector separation and the angle of detection can be varied to influence the sampling volume and polarization ratio of the detected light. To perform the automated scanning/imaging the biotissue samples were placed onto 2D scanning stage. The specific software for stage control and simultaneous measurement of Stokes vector components with the subsequent analysis of the obtained data was also developed. Mapping the Stokes vectors onto the Poincaré sphere allowed visualization and comparison of the polarization states of the detected radiation.

Multiple measurements have been performed with the developed experimental system on a human lung metastasis of basal squamous cell carcinoma embedded in paraffin wax. These samples have a variety of tissue structures, including both healthy and cancerous regions classified by the pathologist.

We demonstrate that circularly polarized light scattered within the tissues is highly sensitive to the presence of cancer cells. Mapping the Stokes vectors of backscattered light on a Poincaré sphere highlight the changes of polarization state. It was shown that the polarization state of the backscattered light from the cancerous samples is located closer to the position of the probing light on the sphere, while the healthy tissues correspond to lower latitudes. Thus, the proposed approach would enable the creation of a tool helping the pathologists in their decisions.

10060-29, Session 6

Optical biopsy of tissue with Mueller polarimetry: modeling and experiments

Tatiana Novikova, Ecole Polytechnique (France); Igor Meglinski, Univ. of Oulu (Finland); Enric Garcia-Caurel, Ecole Polytechnique (France); Alexander Bykov, Univ. of Oulu (Finland); Jean Rehbinder, Stanislas Deby, Jérémy Vizet, Angelo Pierangelo, François Moreau, Ecole Polytechnique (France); Pierre Validire, Abdelali Benali, Brice Gayet, Institut Mutualiste Montsouris (France); Benjamin Teig, André Nazac, CHU Bicêtre (France); Razvigor Ossikovski, Ecole Polytechnique (France)

The rise of optical biopsy as an alternative to classical biopsy is dictated by ongoing technological progress: any type of measurements has to be fast, precise, non-invasive and implemented in-vivo. The use of polarized light for optical biopsy has a long history. As Mueller-Stokes formalism provides the most complete description of polarized light interaction with any type of sample (even depolarizing one) we explored the capabilities of in-house multi-wavelength Mueller imaging polarimeter for the detection of pre-malignancy and malignancy. Our studies were performed with both scattering phantom tissues (in transmission configuration) and specimens of human colon and uterine cervix (in backscattering configuration).

For the interpretation of measurement results we decomposed Mueller matrix of a sample into product of elementary Mueller matrices of homogeneous diattenuator, retarder, and depolarizer. This phenomenological approach does not require the exact solution of Maxwell equations and provides the “effective” values of polarimetric properties of sample.

Exploring differential Mueller matrix formalism for fluctuating medium we showed that depolarization in homogeneous turbid medium varied parabolically with the pathlength of transmitted light, while the standard deviation of elementary polarization properties of medium depends linearly on the concentration of scatterers.

Neither scattering phantoms nor human tissue possessed any measurable diattenuation in backscattering configuration. The polarimetric images of tissue depolarization power, scalar birefringence and orientation of optical axis were compared with the analysis of histological slides. The spectral dependence of depolarization power and scalar birefringence values ascertained the potential of imaging Mueller polarimetry to discriminate healthy and diseased tissue zones.

10060-30, Session 6

Computational model for simulation of sequences of helicity and angular momentum transfer in turbid tissue-like scattering medium *(Invited Paper)*

Alexander Doronin, Yale Univ. (United States); Igor Meglinski, Univ. of Oulu (Finland)

Current report considers development of a unified Monte Carlo (MC) -based computational model for simulation of propagation of Laguerre-Gaussian (LG) beams in turbid tissue-like scattering medium. With a primary goal to proof the concept of using complex light for tissue diagnosis we explore propagation of LG beams in comparison with Gaussian beams for both linear and circular polarization. MC simulations of radially and azimuthally polarized LG beams in turbid media have been performed, classic phenomena such as preservation of the orbital angular momentum, optical memory and helicity flip are observed, detailed comparison is presented and discussed.

10060-31, Session 7

3D Temperature mapping of embedded RF excited MNPs for tumor treatment monitoring

Idan Steinberg, Stanford Univ. (United States); Gil Tamir, Tel Aviv Univ. (Israel); Israel Gannot, Johns Hopkins Univ. (United States) and Tel Aviv Univ. (Israel)

Systemic hyperthermia therapy exploits the fact that cancer cells are more sensitive to elevated temperatures than healthy tissue. Systemic application of hyperthermia externally usually leads to low efficiency treatment. Recently, our group and others have proposed an antibody conjugated magnetic nanoparticles (MNPs) approach to overcome the limitation of systemic hyperthermia. MNPs can bind specifically to the tumor sites, thus delivering internal highly effective targeted hyperthermia. However, such internal mechanism requires more complicated controls and monitoring. This current work presents a deep tissue temperature monitoring method to control hyperthermia effectiveness and minimize collateral damage to surrounding tissues.

A low-frequency narrowband modulation of the RF field used for MNP heating leads to the generation of diffused thermal waves which propagate to the tissue surface and captured by a thermal camera. A Fourier domain, analytical heat transfer model is used for temperature monitoring algorithm. The ill-posed thermal inverse problem is solved efficiently by iterating over the source power until both the amplitude and phase match the recorded thermal image sequence.

The narrow bandwidth thermal stimulation enables acquiring deep signals with high SNR. We show that thermal transverse resolution improves as the stimulation frequency increases even slightly above DC, enabling better heat source transverse separation and margin identification in the case of distributed tumors. These results can be used as a part of an overall image and treat system for efficient detection of tumors, manipulation of MNPs and monitoring MNP based hyperthermia.

10060-32, Session 7

Fast hyper-spectral imaging of cytological samples in the mid-infrared wavelength region

Mark Farries, Gooch & Housego (Torquay) Ltd. (United Kingdom); Jon D. Ward, Gooch & Housego PLC (United Kingdom); Ian D. Lindsay, Univ. of Bristol (United Kingdom); Jayakrupakar Nallala, Univ. of Exeter (United Kingdom); Peter M Moselund, NKT Photonics (Denmark)

Assessment of cells for cancer and other diseases usually requires a biopsy and measurement in a Fourier transform spectrometer, which can take several hours. There is clearly a need for speeding up the measurement process and ideally measuring cells in vivo so that patients can get results quickly with minimal surgery. A prototype mid-ir spectral imaging system has been developed based on a fibre optic super-continuum source that has large spectral brightness. This is coupled in to an acousto-optic tuneable filter that can rapidly scan over a set of wavelengths that are chosen to give a high level of selectivity for a specific skin disease. Light reflected from or transmitted through the skin sample is imaged on a fast ir camera with high resolution so that a large number of individual cells can be measured in a single exposure. The system has the potential to collect an image cube of 80 wavelengths and 300k pixels in 1 second so that cells on living people could be analysed. The system has been evaluated with colon cells over the wavenumber range 2700-3100 cm-1.

10060-33, Session 7

New insight in image registration during Transcranial Optical Vascular Imaging (TOVI)

Vyacheslav Kalchenko, Guillaume Molodij, Yuri Kuznetsov, Weizmann Institute of Science (Israel); Igor Meglinski, Univ. of Oulu (Finland); Alon Harmelin, Weizmann Institute of Science (Israel)

Intravital imaging of brain vasculature through the intact cranium in vivo is based on the evolution of the fluorescence intensity and provides an ability to characterize various physiological processes in the natural context of cellular resolution. In vivo non-invasive functional optical imaging is very often limited due to artifacts associated with the involuntary patients' motions. The computational-based image processing solutions are typically used for corrections of acquired images suffering with motion artifacts.

Transcranial Optical Vascular Imaging (TOVI). TOVI – is a technique that recently was introduced by our group and dedicated to use dynamic fluorescence as one of the contrast for imaging, characterization and quantification of cerebral blood vessels through the intact cranium. This fluorescence imaging technique is also characterized by fast dynamic changes of the forms and patterns over time. Natural movements made during the image recording produce distortions that are unique in each frame. These motions include various jerks, slower drifts, and high frequency tremors perturbing the temporal analysis.

In current work we analyze number of techniques for image registration and focus on a new methodology that utilizes an adaptive Kappa-Omega filtering approach. We demonstrate that the proposed approach is effective for the image stabilization and vibration reduction during TOVI.

10060-34, Session 7

Large area, label-free imaging of extracellular matrix using telecentricity

Michelle Visbal Onufrak, Purdue Univ. (United States); Raymond L. Konger, Indiana Univ. (United States); Young L. Kim, Purdue Univ. (United States)

Subtle alterations in stromal tissue structures and organizations within the extracellular matrix (ECM) have been observed in several types of tissue abnormalities, including early skin cancer and wounds. Current microscopic imaging methods however, lack the ability to accurately determine the extent of malignancy over a large area, due to their limited field of view. In this research we focus on the development of mesoscopic (i.e. between microscopic and macroscopic) biomedical imaging device for non-invasive assessment of ECM alterations over a large, heterogeneous area. In our technology development, a telecentric lens, commonly used in machine vision systems but rarely used in bioimaging, serves as a key platform to visualize alterations in tissue microenvironments in a label-free manner over a clinically relevant area. In general, telecentric imaging represents a simple, alternative method for reducing unwanted scattering or diffuse light caused by the highly anisotropic scattering properties of biological tissue. In particular, under telecentric imaging the light intensity backscattered from biological tissue is mainly sensitive to the scattering anisotropy factor (also known as average cosine of the scattering angle), possibly associated with the ECM. We demonstrate these inherent advantages of telecentric lens systems by imaging precancerous lesions in murine models of photocarcinogenesis, and corroborated with conventional microscopic methods (e.g. second harmonic generation microscopy). Thus, we envision that telecentric imaging could potentially serve for site-specific, tissue-based assessment of stromal alterations over a clinically relevant field of view in a label-free manner, for studying diseases associated with disruption of homeostasis in ECM.

10060-35, Session 7

Microscope systems with unprecedented extended depth of field *(Invited Paper)*

Zengzhuo Li, The Ohio State Univ. (United States); Wei Li, National Eye Institute (United States); Guoqiang Li, The Ohio State Univ. (United States)

Optical microscope has existed for more than three hundred years and has limited depth of field (DoF), e.g., the DoF of an objective lens with NA = 0.4 has a DoF of 3.4 μm in the visible wavelength. We would like to make a revolutionary change in this field by significantly extending the DoF (more than 12 times) using wavefront coding technique. The new microscope permits 3D volume information at one time, and it is extremely useful to observe the samples with dynamic changes. We have custom designed, fabricated, and characterized various phase functions and built both transmissive and reflective microscopes. The features of our work include: (1) The phase plate is simply inserted in between the objective lens and the tube lens, which provides great flexibility in system assembly and switch of the phase plates; (2) Optical design software is used to design the phase plate, which allows the consideration of the aberrations in the system during the optimization process; (3) Custom merit functions have been defined for optimization of the phase plate; (4) Both non-symmetric (general polynomial, general cubic, cubic) and rotationally symmetric phase functions have been adopted ; (5) All the phase plates are fabricated in house. As a result, we have experimentally demonstrated 40-50 μm DoF within which diffraction-limited resolution is maintained, indicating more than 12 times improvement. This is the best result in literature. Imaging of various biomedical samples will be presented. Fluorescence imaging will also be demonstrated.

10060-53, Session 7

Optical measurement of cerebral blood flow during orthostatic manipulation in healthy and diseased populations

David R. Busch, The Children's Hospital of Philadelphia (United States); Clara Gregori-Pla, Martina Giovannella, Igor Blanco, ICFO - Institut de Ciències Fotòniques (Spain); Wesley B. Baker, Ashwin B. Parathasarathy, Univ. of Pennsylvania (United States); Jennifer M. Lynch, Madeline E. Winters, Kobina G. Mensah-Brown, Ann L. McCarthy, The Children's Hospital of Philadelphia (United States); John A. Detre M.D., The Univ. of Pennsylvania Health System (United States); Arjun G. Yodh, Univ. of Pennsylvania (United States); Daniel J. Licht, The Children's Hospital of Philadelphia (United States); Rickson C. Mesquita, Univ. Estadual de Campinas (Brazil); Turgut Durduran, ICFO - Institut de Ciències Fotòniques (Spain)

Posture has been shown to impact cerebral perfusion in healthy and diseased populations. Management of ischemic stroke, for example, focuses on maximizing cerebral blood flow (CBF) to minimize further damage to the ischemic penumbra. However, management strategies are generally empirical in the absence of bedside CBF monitoring. Here we summarize the results of quantitative optical monitoring of CBF during postural manipulation. The results highlight the need for noninvasive bedside monitoring during therapy.

Several previous studies have shown that CBF is generally higher in the supine versus sitting position, consistent with guidelines that ischemic stroke patients should be kept supine as much as possible. Our prior work demonstrated that CBF generally increases with decreased HOB angle, but ~20% of stroke patients demonstrated a paradoxical decrease in CBF at a lower head-of-bed position. Thus, a supine position may be detrimental for these subjects.

We have extended these findings to postural manipulation of 126 healthy and diseased subjects aged 5 to 93 years. Consistent with our prior measurements, we observe lower supine CBF in ~25% of stroke patients and ~6% of controls. Further, we identified a supine CBF 'hysteresis effect'; when subjects were brought back to a supine position, ~80% of subjects showed an increase in CBF compared to initial supine flow.

10060-36, Session 8

Overcoming sampling depth variations in the analysis of broadband hyperspectral images of breast tissue

Esther Kho, Lisanne L. de Boer, Koen K. Van de Vijver, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (Netherlands); Henricus J. C. M. Sterenborg, The Netherlands Cancer Institute (Netherlands) and Academisch Medisch Centrum (Netherlands); Theo J. M. Ruers, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (Netherlands) and MIRA, Univ. Twente (Netherlands)

Worldwide, up to 40% of the breast conserving surgeries require additional operations due to positive resection margins. We propose to reduce this percentage by using hyperspectral imaging for resection margin assessment during surgery.

Spectral hypercubes were collected from 26 freshly excised breast specimens with a pushbroom camera (900-1700nm). Computer simulations of the penetration depth in breast tissue suggest a strong variation in sampling depth (~0.5-10 mm) over this wavelength range. This was confirmed with a breast tissue mimicking phantom study. Smaller penetration depths are observed in wavelength regions with high water and/or fat absorption. Consequently, tissue classification based on spectral analysis over the whole wavelength range becomes complicated. This is especially a problem in highly inhomogeneous human tissue.

We developed a method, called derivative imaging, which allows accurate tissue analysis, without the impediment of dissimilar sampling volumes. A few assumptions were made based on previous research. First, the spectra acquired with our camera from breast tissue are mainly shaped by fat and water absorption. Second, tumor tissue contains less fat and more water than healthy tissue. Third, scattering slopes of different tissue types are assumed to be alike.

In derivative imaging, the derivatives are calculated of wavelengths a few nanometers apart; ensuring similar penetration depths. The wavelength choice determines the accuracy of the method and the resolution. Preliminary results on 3 breast specimens indicate a classification accuracy of 93% when using wavelength regions characterized by water and fat absorption. The sampling depths at these regions are 1mm and 5mm.

10060-37, Session 8

Bio-optic signatures for advanced glycation end products in the skin in streptozotocin (STZ) Induced Diabetes *(Invited Paper)*

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Type 1diabetes (T1D) is an autoimmune disorder that occurs due to the rapid destruction of insulin-producing beta cells, leading to insulin deficiency and the inability to regulate blood glucose levels and leads to destructive secondary complications. Advanced glycation end (AGEs) products, the result of the cross-linking of reducing sugars and proteins within the tissues, are one of the key causes of major complications associated with diabetes such as renal failure, blindness, nerve damage and vascular changes. Non-invasive techniques to detect AGEs are important for preventing the harmful effects of AGEs during diabetes mellitus.

In this study, we utilized multiphoton microscopy to image biopsies taken from control rats and compared them to biopsies taken from streptozotocin (STZ) induced adult male diabetic rats. This was done at two and four weeks after the induction of hyperglycemia (>400 mg/dL) specifically to evaluate the effects of glycation on collagen. We chose to use an in-situ multiphoton microscopy method that combines multiphoton auto-florescence (AF) and second harmonic generation (SHG) to detect the microscopic influence of glycation.

Initial results show high auto-florescence levels were present on the collagen, as a result of the accumulation of AGEs only two weeks after the STZ injection and considerably higher levels were present four weeks after the STZ injection. Future projects could involve evaluating advanced glycation end products in a clinical trial of diabetic patients.

10060-38, Session 8

Design of a modified endoscope illuminator source for spectral imaging of colorectal tissues

Craig Browning, Samuel A. Mayes, Thomas C. Rich, Silas J. Leavesley, Univ. of South Alabama (United States)

The gold standard for locating colonic polyps is a white light endoscope in a colonoscopy. However, polyps smaller than 5 mm are easily missed in a colonoscopy. Modified procedures such as narrow band imaging did not decrease miss rates on small polyps detection either. Spectral imaging is a potential solution to improve the sensitivity and specificity of current procedures by providing the ability to distinguish molecular fluorescence differences in tissues. The goal of this work is to use a LED based endoscopic light source to acquire spectral data of colorectal tissue using colonoscopy, similar to a previous study on lung tissue using microscopy.

A beta-version endoscope light source was developed, by retrofitting a white light endoscope light source (Olympus, CLK-4) with 16 narrow band LEDs. This redesigned, beta-prototype uses high-power LEDs with a minimum output of 50 mW to resolve the low spectral output (0.5 mW) through the endoscope. A mounting apparatus was designed to provide sufficient heat dissipation. Current LED testing is intensity output through the light source and endoscope to determine the flat spectral output for imaging and intensity losses through the endoscope. The imaging process of colon tissue pairs ex vivo (precancerous/normal and cancerous/normal) should result in some practical data.

Optimizing the beta prototype achieved a higher spectral output, allowing the imaging process to begin and potentially determine spectral differences in cancerous and normal tissue. Future work will focus on building a spectral library for the colorectal region and developing a system for in vivo use.

10060-39, Session 8

In vivo measurement of the tissue oxygenation by time-resolved luminescence spectroscopy: Strategies to avoid artefacts associated with photosensitization and photoproducts

Georges Wagnières, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Veronika Huntosova, Ecole Polytechnique Fédérale de Lausanne (Switzerland) and Pavol Jozef ?afárik Univ. in Ko?ice (Slovakia); Denis Horwath, Pavol Jozef ?afárik Univ. in Ko?ice (Slovakia); Emmanuel Gerelli, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

The real-time determination of oxygen partial pressure (pO2) in living biological tissues is of interest for numerous applications, including photodynamic therapy and radiotherapy. It is also linked to fundamental information on cells and tissue respiration.

Important progress has been achieved during past decades to measure pO2 due to the development of time-resolved luminescence measurement methods and the availability of oxygen-sensitive molecular probes. Those include porphyrins, such as aminolevulinic-induced protoporphyrin IX (PpIX), which is a widely used oxygen-sensitive molecule. However, it has drawbacks, which include: i) poor photostability and ii) strong phototoxicity. Since the excitation of PpIX during pO2 measurements leads to the formation of photoproducts, we studied the influence of their luminescence on the PpIX lifetime in solution and in vivo on the Chick's Chorioallantoic Membrane (CAM) model, under various oxygen (between 0 and 155 mmHg) and light (405 nm; 10 J/cm2 or 70-160 J/cm2 for in vivo and solution experiments, respectively) conditions. We demonstrated that the way to avoid perturbations induced by photoproducts luminescence is to either detect PpIX emission in a narrow spectral domain (between 620 and 640 nm), or excite PpIX with light doses smaller than 1 J/cm2.

Since PpIX induces some degree of phototoxicity, we studied the oxygen sensitivity and phototoxicity of an interesting alternative compound, dichlorotris(1,10-phenantroline)-Ruthenium(II) Hydrate (Ru(Phen)) on the same model. We established that the phototoxic threshold of Ru(Phen) is about two orders of magnitude higher than the fluence necessary for pO2 measurements.

10060-40, Session 8

Chemometric endogenous fluorescence for tissue diagnosis

Run Li, Kevin Vasquez, Min Xu, Fairfield Univ. (United States)

Endogenous fluorescence is a powerful technique for probing both structure and function of tissue. Endogenous fluorescence strengths or relative contributions have been used to probe tissue physiological

state and to differentiate normal, dysplasia, and cancer ex vivo and in vivo. Imaging endogenous fluorescence typically requires a confocal microscope with laser excitation owing to its weak strength which hinders its wider applications. Moreover, quantification of the absolute concentration of endogenous fluorophores is much more challenging than measuring the fluorescence intensities.

We present here a chemometric fluorescence microscopy which uses broadband excitation and detection to dramatically increase the signal to noise ratio (SNR) in wide-field fluorescence imaging; and further resolves and maps the absolute concentration of individual fluorescent intrinsic molecules by computational synthesis. Non-negative factorization aided by the spatial diversity is used to learn both the spectral signature and the spatial distribution of endogenous fluorophores from microscopic

fluorescence color images obtained under broadband excitation and detection. The absolute concentration map of individual fluorophores is derived by comparing to the fluorescence from "pure" fluorophores under the identical imaging condition following the identification of the fluorescence species by its spectral signature. This method is then demonstrated by characterizing the concentration map of endogenous fluorophores (including tryptophan, elastin, NADH and FAD) for lung tissue specimens. The absolute concentrations of these fluorophores are found all to decrease significantly from normal, perilesional, to cancerous (squamous cell carcinoma) tissue. Discriminating tissue types using the absolute fluorophore concentration is found to be significantly more accurate than that achievable with the relative fluorescence strength. Quantification of fluorophores in terms of the absolute concentration map is also advantageous in eliminating the uncertainties due to system responses or measurement details, yielding more biologically relevant data, and simplifying the assessment of competing imaging approaches.

10060-41, Session 8

Modulation of the endogenous production of protoporphyrin IX in a yeast-based model organism

Jaroslava Joniova, Emmanuel Gerelli, Georges Wagnières, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

The main aim of this study was to assess the conditions at which a simple yeast (Saccharomyces cerevisiae) – based model organism reproducibly produces maximal levels of protoporphyrin IX (PpIX) after an exogenous administration of its precursor, 5-aminolevulinic acid (ALA), and the ferrous-ion chelator 2,2'-bipyridyl.

We established, in a first step, that the fluorescing porphyrin produced after these administrations was mostly PpIX: fluorescence spectroscopy of the porphyrins produced endogenously in yeast cells resembles that of PpIX in DMSO. In addition, fluorescence lifetimes and the oxygen dependence of the delayed fluorescence lifetime of these porphyrins are very similar to that of PpIX in DMSO. This suggests that PpIX is the main fluorescent compound produced by yeast cells in our conditions. We found that the conditions at which yeast produces the maximal PpIX was a synchronous administration of 5 ?M ALA and 1 mM 2,2'-bipyridyl.

Such a simple model is of high interest to study basic mechanisms involved in the mitochondrial respiration since PpIX, which is produced in this organelle, can be used as oxygen sensor, or to optimize photodynamic therapy and photodiagnosis based on PpIX. In particular, a version of this model loaded with appropriate amounts of light absorbing and scattering particles could be used as phantom to mimic tumors containing PpIX, a useful tool to optimize the spectral and radiometric design of certain cancer photodetection set-ups. This explains why we have also characterized the absorption and scattering properties of this model at different wavelengths of interest for these applications.

10060-42, Session 8

Structured interferometry features in femtosecond supercontinuum: towards better understanding of supercontinuum for bio applications *(Invited Paper)*

Debabrata Goswami, Sirshendu Dinda, Soumendra N. Bandyopadhyay, Indian Institute of Technology Kanpur (India)

Near-threshold interferrometry features seen in supercontinuum generation process have been explored We argue that these structured interferometry features in femtosecond supercontinuum at laser intensities near to supercontinuum generation threshold arise from the coherent superposition of supercontinuum generated from different sources of supercontinuum. This results as the incident pulse split into two daughter pulses. Increase in input pulse energy creates several more temporal pulse fragments and disrupts interference resulting in the typical feature of continuous broad supercontinuum. Such an understanding of supercontinuum generation process is critical to the use of supercontinuum as the light source for use in understanding the time dynamics and imaging of bio systems. Much of the coherent oscillation features seen in ultrafast time dynamics may arise from such inherent features of the probing or exciting source rather than light interactions with the system. Thus, such an understanding is important to ensure that the measurements with supercontinuum as light source are made either under conditions where the supercontinuum source features are either completely known or are generated under conditions where they are devoid of such issues.

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10061-1, Session 1

Hybrid carbon nanotube-polymer scaffolds for cardiac tissue regeneration *(Invited Paper)*

Samad Ahadian, Locke Davenport-Huyer, Univ. of Toronto (Canada); Mehdi Estili, National Institute for Materials Science (Japan); Bess Yee, Nathaniel Smith, Zhensong Xu, Yu Sun, Milica Radisic, Univ. of Toronto (Canada)

Due to insufficient number of heart transplants and limited regenerative ability of heart tissues, cardiac tissue engineering has emerged to restore or regenerate the structure and function of native cardiac tissues. Scaffolds play a major role to fabricate functional cardiac tissues providing structural support, biodegradation, and cell affinity in tissues. However, currently used scaffolds in cardiac tissue regeneration lack electrical conductivity and high mechanical properties. Here, we propose the use of carbon nanotubes (CNTs) to enhance electrical and mechanical properties of polyester scaffolds. Swelling of polyesters was mediated after adding CNTs. CNTs also increased electrical conductivity of polyesters. Hybrid CNT-polyester materials showed no cytotoxicity to cardiomyocytes. More importantly, functional properties of cardiac tissues using CNT-polyester scaffolds were improved compared to those made of pure polyester scaffolds.

10061-2, Session 1

Fabrication of biomimetic fog-collecting surface micropatterns using femtosecond lasers

Elisabeth Kostal, Sandra Stroj, Stephan Kasemann, FH Vorarlberg (Austria); Victor V. Matylitsky, Spectra-Physics Rankweil (Austria); Matthias Domke, FH Vorarlberg (Austria)

The exciting functionalities of natural superhydrophobic and superhydrophilic surfaces, e.g. the extreme water repellency of the lotus flower, served as inspiration for a variety of biomimetic designs. In recent years it was successfully demonstrated that both hydrophobic and hydrophilic surfaces can be fabricated by laser machining. In contrast, little attention has been paid to combine both extreme wetting states to micropatterns. However, as the example of the fog-collecting Namib desert beetle shows, such micropatterns offer exciting possibilities for the design of biomedical and microfluidic devices.

In this study we present an application of the newly developed, patent-pending ClearSurfaceTM technology from Spectra-Physics® based on femtosecond laser machining The ClearSurfaceTM process in combination with an industrial fs laser Spirit® 1040-4 SHG allows the fast and flexible fabrication of superhydrophilic micropatterns (contact angle < 5°) on a superhydrophobic background (contact angle > 150°). The innovative process enables to apply these wetting properties to nearly all kind of substrates and the resulting contact angle can be precisely controlled by adjusting the laser parameters.

Following the example of the fog-collecting elytra of the Namib desert beetle, superhydrophilic spots on a superhydrophobic background were generated on Pyrex wafers. The water collection efficiency was tested in an artificial nebulizer setup. The experiments showed that the surface micropatterns enhance the collection efficiency by nearly 40% compared to smooth surfaces. The fog droplets are captured on the superhydrophilic micropattern, grow to drops of a size of about 5 mm until they are detached by gravity. The superhydrophobic areas induce a fast drop removal which increases the collection efficiency.

10061-3, Session 1

Rapid structuring of proteins on filter paper using lithography

Tobias M. Nargang, Frederik Kotz, Nico Keller, Dorothea Helmer, Bastian E. Rapp, Karlsruher Institut für Technologie (Germany)

Microfluidic paper based analytical devices (?PADs) are simple and cost efficient and can be used everywhere without the need for a high standard laboratory for obtaining a readout. These devices are thus especially suited for the developing world or crisis regions. To fabricate a bioanalytical test, certain biomolecules like proteins or antibodies have to be attached to paper strips. Common immobilization methods often rely on uncovalent, unoriented attachment which leads to reduced bioactivity of the immobilized species. Specific Immobilization of biomolecules on surfaces still poses a great challenge to biochemical research and applications.

We propose a method for the specific immobilization of biomolecules on functionalized filter paper using a maskless projection lithography setup. The paper was functionalized either by applying an adhesive protein coating or by covalent attachment of methacrylate groups. Fluorescently labelled biomolecules were attached by exploiting the formation of radical species upon bleaching of the fluorophore. A custom made maskless photo-lithography setup was used to produce microscale biomolecule greyscale patterns. This way, proteins, enzymes and antibodies were covalently and specifically immobilized onto paper strips. Protein patterns were visualized by antibody staining, enzyme patterns were tested for bioactivity by substrate conversion with colorimetric readout and antibody patterns were visualized by an antigen/antibody sandwich.

This method enables the creation of complex, highly specific bioactive protein patterns and greatly facilitates the production of ?PADs.

10061-4, Session 1

Erasable microfluidic channels based on photoelectrowetting of ZnO films

Khaled M. Al-Aribe, Abu Dhabi Univ. (United Arab Emirates); George K. Knopf, Western Univ. (Canada)

Photoelectrowetting can be used to drive droplets of liquid along reconfigurable paths on a microfluidic chip using controlled optical signals. These electrostatically activated surfaces along the desired path eliminate the need for precision molded microchannels or mechanically actuated valves and pumps. The photoelectrowetting effect exploits the surface tension of the droplet to maintain its volume during transportation, and the photoelectric properties of the dielectric substrate surface are used to induce reversible fluidic flow. The active light-driven substrate is created by depositing zinc-oxide (ZnO) on an indium-tin oxide (ITO) coated glass. In addition, this substrate is coated from the ZnO side with Ruthenium-based dye (N719) to adjust and maximize its absorbability to UV light band. The optical beam triggers two forces that enable the droplet to be transported along the substrate. The first arises from the induced hydrophobicity gradient formed across the droplet contact area with the substrate surface. Exposing the ZnO film to the UV beam, influences the surface's electric potential which in order changes the droplet's contact angle and the associated hydrophobicity. Once the hydrophobicity gradient is generated, the droplet starts moving in the direction of the wetting zone. The second force is created by the same optical input where the absorbed UV light generates a photoelectric potential which is processed piezo-electrically by the ZnO film to generate the erasable micro-channel that guides the droplet movement. Preliminary experiments are summarized and future work is discussed.

10061-5, Session 1

Laser induced forward transfer technique for the immobilization of biomaterials in biosensors applications

Symeon Papazoglou, Marianeza Chatzipetrou, Maria Massaouti, Ioanna Zergioti, National Technical Univ. of Athens (Greece)

Laser Induced Forward Transfer (LIFT) is a direct write technique, able to create micropatterns of biomaterials on sensing devices. In this conference we will present a new approach using LIFT for the printing and direct immobilization of biomaterials on a great variety of surfaces, for bio-sensor applications. The basic requirement for the fabrication of a biosensor is to stabilize a biomaterial that brings the physicochemical changes in close proximity to a transducer. In this direction, several immobilization methods such as covalent binding and crosslinking have been implemented. The presence of the additional functionalization steps in the biosensors fabrication, is among the main disadvantages of chemical immobilization methods. Our approach employs the LIFT technique for the direct immobilization of biomaterials, either by physical adsorption or by covalent bonding of the biomaterials. The physical adsorption of the biomaterials, occurs on hydrophobic or super-hydrophobic surfaces, due to the transition of the wetting properties of the surfaces upon the impact of the biomaterials with high velocity. The unique characteristic of LIFT technique to create high speed liquid jets, leads to the penetration of the biomaterial in the micro/nano roughness of the surface, resulting in their direct immobilization, without the need of any chemical functionalization layers. Moreover, we will also present the direct immobilization of biomaterials on Screen Printed Electrodes, for enzymatic biosensors, with a limit of detection (LOD) for catechol at 150 nM, and protein biosensors, used for the detection of herbicides, with an LOD of 8-10 nM.

10061-6, Session 2

Ion mobility spectrometry-based chemical detection systems for medicine, agriculture and security *(Invited Paper)*

Cristina Davis, Univ. of California, Davis (United States)

Precision chemical sensors can potentially play an important role in many industry sectors. Performance parameters such as sensitivity and specificity are critical to match candidate sensors to their best applications, and operating parameters such as temperature and pressure ranges for an application must also match appropriately. One important category of chemical sensors has gained attention due to performance across a broad range of operating conditions. Ion mobility spectrometry (IMS) devices distinguish chemicals based on mobility in an electric field, and there are variations of IMS based on tailored configurations, geometries and size of the instrument. Differential mobility spectrometry (DMS) is an attractive sub-set of these devices, and they exist in a microfabricated format amenable to small-footprint systems. While IMS and DMS sensors alone have impressive performance characteristics, their performance can be enhanced by incorporating them into system-level platforms with other miniature components that perform specific functions. My research group designs next-generation IMS-like sensor systems that incorporate new modular concepts for sampling, pre-concentration, pre-separation, detection and real-time rapid data analysis software. These broad platform system concepts have important trace chemical detection applications in many industries including medicine, agriculture and defense.

10061-8, Session 2

Investigation of the capillary flow through open surface microfluidic structures

Ahmed Taher, IMEC (Belgium) and KU Leuven (Belgium); Benjamin Jones, Paolo Fiorini, IMEC (Belgium); Liesbet Lagae, IMEC (Belgium) and KU Leuven (Belgium)

Capillary microfluidics is attractive for many applications including point of care medical diagnostics. For applications that need chemical or biological materials spotted or dried at the bottom of microfluidic channels after device fabrication, it is often more practical to have open surface devices (i.e. without cover). Capillary driven flow is deemed essential for such devices as pressure driven flow cannot be used and gravitational forces are small at the typical device length scales. However, the dynamics of capillary driven flow in open surface devices have not been well studied for many geometries of interest.

In this paper, we investigate capillary flow in right angle and inclined bifurcations, backward facing steps and T-junctions. These device geometries were studied analytically, experimentally and using numerical simulations. We developed mathematically a flow condition for whether flow will proceed or stop in each device structure. For instance, a relation between the contact angle, channel width and bifurcation inclination angle was developed analytically to determine the condition for capillary flow to proceed through an open surface channel with a bifurcation.

Test devices were designed to meet or fail the analytical criteria. The test devices were fabricated in silicon and tested using fluorescently dyed water. The images and videos of the capillary flow under the microscope were captured using a high speed camera. Numerical simulations were also performed using the volume of fluid model in Ansys Fluent software. The experimental and numerical simulation results confirmed the developed conditions for each of the device structures.

10061-9, Session 2

A 3D particle focusing device based on tightly curving 3D microchannels

Petra Paiè, Francesca Bragheri, CNR-Istituto di Fotonica e Nanotecnologie (Italy); Dino Di Carlo, Univ. of California, Los Angeles (United States); Roberto Osellame, CNR-Istituto di Fotonica e Nanotecnologie (Italy)

Particle focusing is an important functionality useful in a wide set of biological applications. Nevertheless, it is still challenging to realize it in microfluidics, especially in a low pressure system, because of the intrinsic 2D nature of standard microfluidic devices; long channels or complicated device geometries with several lateral channels are usually needed to avoid this limitation. In this work we present a compact microfluidic chip, which is capable to perform 3D particle focusing at high flow rates, thanks to the superposition of inertial focusing and intense Dean flow in tightly curving 3D channels. The device layout comprises alternating helices and straight channel sections permitting particle focusing with driving pressures < 1 bar due to the compactness of this chip. The complete device optimization and validation is presented, demonstrating the capability of the chip to effectively focus the sample using a single inlet, with no need of additional lateral channels that complicate the sample processing procedure. Moreover, the device layout facilitates a parallelization of channels, with the positive consequence to speed up the time needed to process the sample. Femtosecond laser micromachining followed by chemical etching is used to fabricate the device. This technique is a two-step process that permits fabrication of 3D structures in fused silica substrates and it is a fundamental tool to obtain 3D helices in the substrate. A surface fabrication approach has been used to avoid tapered channels. We envisage the use of this chip for high speed flow cytometer applications.

10061-10, Session 2

AC electrothermal technique in microchannels

Alinaghi Salari, Univ. of Calgary (Canada); Maryam Navi, Ryerson Univ. (Canada); Colin Dalton, Univ. of Calgary (Canada)

Electrokinetic techniques have a wide range of applications in droplet and fluid manipulation systems. In general, they can be categorized into different subgroups including electroosmosis, electrothermal, electrophoresis, dielectrophoresis, etc. The AC electrothermal (ACET) technique has been shown to be very effective in applications which involve high conductivity fluids, such as blood, which are typically used in biomedical applications. In the past few years, the ACET effect has received considerable attention. Unlike AC electroosmosis ACEO, the ACET effect shows plateaus in force in a wide frequency range. In other words, with electrothermal force, velocity is more steady and predictable at different frequencies, compared to ACEO and dielectrophoresis (DEP). Although electrothermal microflows form as a result of heating in the fluid, due to high conduction of heat to the ambience, the temperature rise in the fluid is not so high as to threaten the nature of the biofluids. The average temperature rise resulting from the ACET effect is below 5 K. In order to generate high strength AC electric fields, microfabricated electrode arrays are commonly used in microchannels. For pumping applications, it is essential to create asymmetry in electric field, typically by having asymmetrical electrode pairs. There is no defined border between many electrokinetic techniques, and as such the point where ACET interferes with other electrokinetic techniques is not clear in the literature. In addition, there have been comprehensive reviews on micropumps, electrokinetics, and their subcategories, but the literature lacks a detailed up-to-date review on electrothermal microdevices. In this paper, a brief review is made specifically on electric fields in ACET devices, in order to provide an insight for the reader about the importance of this aspect of ACET devices and the improvements made to date

10061-11, Session 3

Nanoscale patterning of biopolymers for controlled microfluidic behavior and drug release

Akshit Peer, Iowa State Univ. of Science and Technology (United States) and Ames Lab. (United States); Rabin Dhakal, Iowa State Univ. of Science and Technology (United States); Rana Biswas, Iowa State Univ. of Science and Technology (United States) and Ames Lab. (United States); Jaeyoun Kim, Iowa State Univ. of Science and Technology (United States)

It is of great interest to pattern biomaterials on the nanoscale, to control diverse biomedical functions such as cell growth, microfluidic behavior and drug release. We utilize a rapid soft lithographic technique to nanopattern poly (L-lactic acid) (PLLA) - a biodegradable/biocompatible polymer. We fabricated PDMS molds with periodic arrays with pitch of ~750 nm, and transfer their inverse patterns to PLLA surfaces through solution casting. Drop casting was superior to nanoimprinting in preserving the fidelity of nanoscale features.

We investigate the nanopattern's impact on the release of sirolimus, an immuno-suppressant agent, coated on the PLLA surface. Samples of the drug released into a saline solution were collected from 1 hour to >12 days. The drug release rate was measured with high performance liquid chromatography/mass spectrometry and compared with flat reference samples.

The PLLA surfaces nanopatterned with 750 nm pitch nanocup or nanocone arrays exhibit drug release rates significantly (25-30%) lower than that of the flat surface, which is counter-intuitive given the nanopattern-induced increase in their surface areas. Based on diffusion and microfluidic meniscus curvature minimization analyses, we attribute the decreased drug release rate to the incomplete wetting of the nanopatterned surface by the fluid. The wetting behavior turns out to depend strongly on the shape of the nanopattern. These results provide new insights on how surface nanopatterning of biomaterials can modify the surface characteristics and tailor the release kinetics of therapeutic agents coated on it for controlled drug elution. These findings have potential applications to biomedical microsystems and therapeutics.

10061-12, Session 3

3D printed molds for facile PDMS casting of microfluidic channels

Phillip Nguyen, Francisco J. Tovar-Lopez, Kate Fox, Arnan Mitchell, RMIT Univ. (Australia)

Microfabrication of planar microfluidic channels has long been performed using soft lithography processes. Soft lithography has remained in use since the beginning of microfluidics as a field of research, and are derivative of semiconductor industry technology. Fabrication involving soft lithography is a manual process, time-consuming and expensive, especially when multi-layer features are required. The recent introduction of high-resolution 3d printing has enabled the direct printing of microfluidic devices and molds which circumvent previous limitations. In this work, ready-to-use molds were printed using a high-resolution industrial stereolithography (SLA) system, which were used to directly fabricate polydimethylsiloxane (PDMS) microfluidic chips. In addition, a number of test structures were also used to evaluate the ability to produce microfluidic structures. Surface characterization, in addition to scanning electron microscopy (SEM) was performed to gauge expected performance characteristics. Physical perturbations that arose as side-effects of the stereolithography photopolymerization process are also described, as their behavior dictates minimum tolerances and the effects of curing artefacts. Resulting performance comparison using microparticle image velocimetry between sets of bifurcated channels, fabricated through the SLA method and conventional soft lithography using acetate photomasks revealed that the printed channels supported flows in much the same way.

10061-14, Session 3

Fast and cheap fabrication of molding tools for polymer replication

Christiane Richter, Karlsruher Institut für Technologie (Germany); Nadine Kirschner, Karlsruhe Institute of Technology (Germany); Matthias Worgull, Bastian E. Rapp, Karlsruher Institut für Technologie (Germany)

Polymer replication is a prerequisite for low-cost microstructure components for consumer and end user market. The production of cost-effective microstructures in polymers requires metal molding tools which are often fabricated by direct structuring methods like milling or laser machining which are time-consuming and cost-intensive.

We present an alternative fabrication method based on replication processes which allows the cheap (~50€) replication of complex microstructure into metal within short periods (~12h). The process comprises the following steps:

1. Generation of the microstructure in a photoresist via lithography.

2. Casting of the structure into a high-temperature silicone which serves as original mold for creation of the metal molding tool.

3. Melting of an eutectic alloy of Sn, Ag and Cu with a melting point of 217°C under light pressure directly inside of the silicone places within an oven. After cooling to room temperature the metal molding tool can be used for polymer replication into conventional thermoplastic polymers.

As a first example we structured polymethylmethacrylate foils with a

thickness of 1mm via hot embossing. Feature sizes of 100μm could be replicated with high fidelity.

Currently the lifetime of a molding tool, i.e., the maximum number of replications which can be done is the cost-determining factor of industrial replication processes. If the replication tool itself is manufactured via replication, this problem is circumvented as the tool can be regenerated. We believe that this method can be a game changer in fabricating molding tools not just for microstructuring but for any type of polymer replication.

10061-15, Session 4

3D printed disposable optics and lab-on-a-chip devices for chemical sensing with cell phones *(Invited Paper)*

German Comina, Anke Suska, Daniel Filippini, Linköping Univ. (Sweden)

Digital manufacturing (DM) offers fast prototyping capabilities and great versatility to configure countless architectures at affordable development costs. Autonomous lab-on-a-chip (LOC) devices, conceived as only disposable accessory to interface chemical sensing to cell phones, require specific features that can be achieved using DM techniques.

Here we describe stereo lithography 3D printing (SLA) of optical components and unibody-LOC (ULOC) devices using consumer grade printers. ULOC devices integrate actuation in the form of check-valves and finger pumps, as well as the calibration range required for quantitative detection. Coupling to phone camera readout depends on the detection approach, and includes different types of optical components.

Optical surfaces can be locally configured with a simple polishing-free post-processing step, and the representative costs are 0.5 US$/device, same as ULOC devices, both involving fabrication times of about 20 min.

10061-16, Session 4

Implementation of a protocol for assembling DNA in a Teflon tube

Edmond Walsh, Alex Feuerborn, Peter Cook, Univ. of Oxford (United Kingdom)

In this paper we demonstrate the use of a new approach to merging and mixing drops, and the transport of molecules between drops. The approach only requires a re-usable Teflon tube attached to a pump and at least three immiscible fluids. Using these three immiscible fluids we exploit interfacial tension, rather than geometry as is common microfluidics using channel networks, to create fluidic architectures that enable merging of, and advection between drops. The approach is demonstrated with a multi-step protocol for the creation of ligated double-stranded DNA molecule using “Gibson mix” chemistry. This protocol is initially demonstrated through the merging and mixing of five drops, followed by a thermal cycle, to assembly Oligonucleotides (Oligos) to form ligated double-stranded DNA molecules. Then, with the use of magnetic beads we implement a more complete protocol within a single Telfon tube with a single inlet. The implemented protocol consists of nine wash steps, merging of four drop, and transport of reagents between twelve drops, followed by a thermal cycle and deposition of final purified solution into eppendorf for downstream analysis. Gel electrophoresis is used to confirm successful ligation of four Oligo’s resulting in ligated double-stranded DNA molecules of expected molecular weight.

10061-17, Session 4

Microfluidic particle manipulation using high frequency surface acoustic waves

Ye Ai, Singapore Univ. of Technology & Design (Singapore)

Precise manipulation of particles and biological cells is an essential process in various biomedical research fields, industrial and clinical applications, which remains a very active research area in microfluidics. Among various force fields applied for microfluidic manipulations, acoustic waves have superior propagating properties in solids and fluids, which can readily enable non-contact cell manipulation in long operating distances. In addition, acoustic fields are advantageous to high power laser beams for non-contact optical tweezing in terms of biocompatibility, throughput and setup simplicity. Exploiting acoustic waves for fluid and cell manipulation in microfluidics has led to a newly emerging research area, acoustofluidics. In this presentation, I will talk about particle and cell manipulation in microfluidics using high frequency surface acoustic waves (SAW). In particular, I will discuss a unique design of a focused IDT (FIDT) structure, which is able to generate a highly localized SAW field on the order of 20 μm wide. This highly focused acoustic beam has an effective manipulation area size that is comparable to individual micron-sized particles. Here, I demonstrate the use of this highly localized SAW field for single particle level sorting using sub-millisecond pulses and selective capture of particles. Based on our research studies on acoustic particle manipulation, I envision that the merging of acoustics and microfluidics could enable various particle and cell manipulations needed in microfluidic applications.

10061-18, Session 4

Rapid prototyped Microfluidic PCR platform to detect mutations associated with multiple drug resistance tuberculosis (MDR-TB)

Smrithi Ajit, Puneeth S B, Bharat Sesham, K.N. Mohan, Sanket Goel, Birla Institute of Technology and Science, Pilani (India)

Today, Polymerase Chain Reaction plays an indispensible role in the field of biomedical research. Its inherent ability to exponentially amplify sample DNA has proven useful for the identification of virulent pathogens like those causing Multiple Drug-Resistant Tuberculosis (MDR-TB). Mutations that assist the pathogen to survive the existing array of drugs used for TB treatment and the difficulty of early detection in preventing its spread make its identification and curtailment an onerous challenge. The intervention of Microfluidics technology has revolutionized the concept of PCR from being a laborious and time consuming process into one that is faster, easily portable and capable of being multifunctional. The Microfluidics based PCR outweighs its traditional counterpart in terms of flexibility of varying reaction rate, operation simplicity, need of a fraction of volume and capability of being integrated with other functional elements. The scope of the present work involves the development of a real-time continuous flow microfluidic device, fabricated by 3D printing-governed rapid prototyping method, leading to an automated and robust platform to process multiple DNA samples for detection of MDR-TB-associated mutations. The thermal gradient characteristic to the PCR process are placed using micro-heater units appropriate to the microfluidic environment fully monitored and controlled by Arduino-based controller. The process efficiency achieved in the microfluidic environment in terms of output per cycle will be on par with the traditional PCR and earn the additional advantage of being faster besides improving the chances of identification of patients carrying MDR-TB and minimizing the handling.

10061-19, Session 5

System-level integration of active silicon photonic biosensors *(Invited Paper)*

Loic Laplatine, Edison Luan, Carter Fang, Osama Al'Mrayat, Shayan Rezaie, Karen C. Cheung, The Univ. of British Columbia (Canada); Yonathan Dattner, Luxmux Technology Corp. (Canada); Lukas Chrostowski, The Univ. of British Columbia (Canada)

Biosensors based on silicon photonic integrated circuits have attracted a growing interest over the last decade due to the possibility of low-cost mass production provided by semiconductor foundries. This activity has led to the development of a myriad of devices such as ring or Bragg grating resonators reaching relevant sensitivities for biomedical diagnosis. However, while most work has focused on sensor devices, the practical integration of silicon photonic biochips as part of a complete system has received less attention. Microfluidic and optical integration generally relies on relatively large chips (~cm2) connected to smaller fluidic gaskets while keeping parts of the chip accessible for electrical and optical coupling. Given the small footprint of ring resonators, most of the chip area is "lost" and only serves as a mechanical support, which negatively impacts the unit cost. In this work, we developed a novel system-level architecture where the chip size can be as small as 1 mm2, while allowing complex and dense microfluidic, optical and electrical integration. By optimizing an epoxy encapsulation process, we pattern electrical interconnects by both photolithography and aerosol jet printing. As for the microfluidic system, it is no longer limited in size and can be simply sealed by mechanical pressure. Finally, optical fiber coupling is seamlessly integrated as part of the fluidic gasket by means of laser micromachining. This packaging method requires only a single photolithography step and paves the way toward inexpensive cartridge containing active silicon photonic biosensors for biomedical and environmental monitoring

10061-20, Session 5

Light field 3D endoscope based on electrowetting lens array

Jin Su Lee, Gyu Suk Jung, Yong Hyub Won, KAIST (Korea, Republic of)

In this study, we propose light field 3D endoscope using the electrowetting micro lens array. Compared to conventional light field endoscope technology, the electrowetting micro lens array are not only switchable between 2D and 3D, but also adjusts the focal length to capture the varying images and control the diopter sufficiently fast (~few ms). The electrowetting micro lens array has diameter 300um and diopter -500D ~ 400D, which is an appropriate to get an endoscopic image. We also compare with light field 3D endoscope using a fixed focus micro lens array and our proposed light field 3D endoscope under the same condition. To achieve the electrowetting micro lens array, Al2O3 layer, SiO2 layer and Teflon layer are deposited on the silicon through hole substrate. In this study, we focus on the electrowetting lens array fabrication and feasibility of a light field 3D system based on the electrowetting micro lens array, accordingly we do not assemble the whole system in the real endoscope. Although it is performed on the optical stage, we successfully capture a light field images of several objects and reproduce a 3D image. Hereafter research, we will apply extended depth-of-field algorithm in our technology to improve the 3D image resolution and depth of field.

10061-21, Session 5

Cylindrical liquid lens with tunable-focus

Huanchen Chen, Univ. of Alberta (Canada); Nima Tabatabaei, Alidad Amirfazli, York Univ. (Canada)

Combining the principles of optics and interfacial phenomenon, in this study we present a novel way to create a tunable-focus cylindrical liquid lens with liquid bridge between two narrow surfaces. Due to the surface edge effect, the interface of the bridge between two long solid surfaces is shown to be able to serve as a tunable-focus cylindrical liquid lens. The curvature of the bridge interfaces (k_1^a) and hence the focal distance of the lens can be manipulated by varying either the height of the bridge (H) or the volume of the liquid (V), allowing the bridge to serve as either diverging or converging lens. With the increase of H, the curvature of the bridge interface which governs the lens focal distance decreases monotonically. Mathematical modeling as well as experiments suggests a critical bridge height exists where k_1^a is zero. When H is larger than Hc, k_1^a is negative (concave bridge) and when H is smaller than Hc, k_1^a is positive (convex bridge). The bridge volume can also affect the performance of the liquid lens. More interestedly, it is shown that above a certain V, k_1^a of the bridge is always positive and no Hc can be found (i.e., the bridge always serve as a diverging lens). Consequently, a small volume bridge is suitable for one to create a liquid lens with small height but large range of the focal distances, while a large volume bridge is suitable to create a liquid lens with larger height but smaller range of the focal distance.

10061-22, Session 5

An optofluidic approach for Gold nanoprobes based-cancer theranostics

Nishtha Panwar, Peiyi Song, Chengbin Yang, Ken-Tye Yong, Swee Chuan Tjin, Nanyang Technological Univ. (Singapore)

Suppression of overexpressed gene mutations in cancer cells through RNA interference (RNAi) technique is a therapeutically effective modality for oncogene silencing. In general, transfection agent is needed for siRNA delivery. Also, it is a tedious process to analyze the gene transfection using current conventional flow cytometry systems. Therefore, there are two urgent challenges that we need to address for understanding and monitoring the delivery of siRNA to cancer cells. One, non-toxic, biocompatible and stable non-viral transfection agents need to be developed and investigated for gene delivery in cancer cells. Two, new, portable optofluidic methods need to be engineered for determining the transfection efficiency of the nanoformulation in real time. First, we demonstrate the feasibility of using gold nanorods (AuNRs) as nanoprobes for the delivery of Interleukin-8 (IL-8) siRNA in a pancreatic cancer cell line- MiaPaCa-2. An optimum ratio of 10:1 for the AuNRs–siRNA nanoformulation required for efficient loading has been experimentally determined. Promising transfection rates (≈88%) of the nanoprobe-assisted gene delivery are quantified by flow cytometry and fluorescence imaging, which are higher than the commercial control, Oligofectamine. The excellent gene knockdown performance (over 81%) of the proposed model support in vivo trials for RNAi-based cancer theranostics. Second, we present an optical fiber-integrated microfluidic chip that utilizes simple hydrodynamic and optical setups for miniaturized on-chip flow cytometry. The chip provides a powerful and convenient tool to quantitatively determine the siRNA transfection into cancer cells without using bulky flow cytometer. These studies outline the role of AuNRs as potential non-viral gene delivery vehicles, and their suitability for microfluidics-based lab-on-chip flow cytometry applications.

10061-23, Session 6

A portable fluorescent sensing system using multiple LEDs *(Invited Paper)*

Young-Hoon Shin, Louisiana State Univ. (United States); Jonathan Z. Barnett, Univ. of Louisville (United States); M. Teresa Gutierrez-Wing, Louisiana State Univ. (United States); Kelly A. Rusch, Lousiana State Univ. (United States); Jin-Woo Choi, Louisiana State Univ. (United States)

Fluorescence-based sensing is a widely used technique in many sensing applications due to the advantages of non-contact, simple configuration, and fast speed. However, a portable fluorescent sensing system for on-site use has been a challenge in practical applications. In this work, we present a portable fluorescent sensing system using multiple LED excitation lights for multianalyte detection. Excitation lights are controlled by an integrated electronic circuit with a microcontroller and signals from target analytes are measured by a highly sensitive silicon photomultiplier. The system consists of a display with control/selection buttons, battery, data storage card, and sample loading tray. A PDMS microfluidic chip is loaded with an aliquot volume of sample. The housing components are made from ABS plastic using a 3D printer. We tested the developed system for the detection of key pigments (chlorophyll a and phycocyanin) in microalgal co-culture species to demonstrate multianalyte detection capability. The system was able to distinguish the species and the concentration of each species. Since the components used in the developed system are not readily available and not expensive, the portable fluorescent system could be a viable option for on-site use and in resource-limited settings.

10061-24, Session 6

Design of portable, point-of-care microfluidic cytometry devices for medical diagnostics

James F. Leary, Aurora Life Technologies (United States)

Design of portable microfluidic flow/image cytometry “reader/disposable chip” based devices for point-of-care (POC) cell-based medical diagnostics requires careful design in terms of power requirements and weight to allow for realistic portability either as POC medical diagnostics in doctors’ offices or in the field (where there is the additional requirement of a telecommunications system). It needs to work with whole peripheral blood directly into the chip where it is mixed directly with reagents. Weight/power requirements dictate use of super-bright LEDs with top-hat excitation beam architectures and very small silicon photodiodes or nanophotonic sensors which can be powered by batteries. Signal-to-noise characteristics can be greatly improved by appropriately pulsing the LED excitation sources and sampling and subtracting noise in between excitation pulses. The requirements for basic computing, imaging, GPS and basic telecommunications can be simultaneously met by use of conventional or satellite cellphone technologies which become part of the overall device. Data analysis should be fully automated based on barcoded chips. Microfluidic cytometry also requires judicious use of small sample volumes and appropriate statistical sampling for adequate statistical significance to permit real-time (typically less than 20 minutes) medical decisions for patients at the physician’s office or in the field. It also provides a more reasonable alternative to conventional tubes of blood when sampling geriatric and newborn patients for whom a conventional peripheral blood draw can be problematical. One or two drops of blood obtained by pin-prick should be able to provide statistically meaningful results for use in making most real-time medical decisions.

10061-25, Session 6

Chemiluminescence generation and detection in a capillary-driven microfluidic chip

Charlotte Ramon, Yuksel Temiz, Emmanuel Delamarche, IBM Research - Zürich (Switzerland)

The use of microfluidic technology represents a strong opportunity for providing sensitive, low-cost and rapid diagnosis at the point-of-care and such a technology might therefore support better, faster and more efficient diagnosis and treatment of patients at home and in healthcare settings, both in developed and developing countries. In this work, we consider luminescence-based assays as an alternative to well-established fluorescence-based systems because luminescence does not require a light source or expensive optical components, and is therefore a promising detection method for point-of-care applications. Here, we show a proof-of-concept of chemiluminescence (CL) generation and detection in a capillary-driven microfluidic chip for potential immunoassay application. We employed a commercial acridan-based reaction, which is catalyzed by horseradish peroxidase (HRP). We investigated CL generation under flow conditions using a simplified immunoassay model where HRP is used instead of the complete sandwich immunocomplex. First, CL signals were generated in a capillary microfluidic chip by immobilizing HRP on a polydimethylsiloxane (PDMS) sealing layer using microcontact printing and flowing CL substrate through the hydrophilic channels. CL signals were detected using a compact (only 5?5?2.5 cm3) and custom-designed scanner, which was assembled for less than $30 and comprised a 128?1 photodiode array, a mini stepper motor, an Arduino microcontroller, and a 3D-printed housing. Next, a 30-μm-deep microfluidic chip was fabricated where a large number of HRP enzymes were localized on 5 μm beads that were trapped in two separate parts of the microchannel by means of capillary assembly and CL signals were detected from these locations.

10061-26, Session 6

Multipath trapping dynamics of nanoparticles towards an integrated waveguide with a high index contrast

Hao Tian, Tianjin Univ. (China) and Massachusetts Institute of Technology (United States); Lionel C. Kimerling, Jurgen Michel, Massachusetts Institute of Technology (United States); Guifang Li, CREOL, The College of Optics and Photonics, Univ. of Central Florida (United States) and Tianjin Univ. (China); Lin Zhang, Tianjin Univ. (China) and Massachusetts Institute of Technology (United States)

Optical trapping and manipulation of nanoparticles in integrated photonics devices has attracted increasingly more attention in biophotonics, sensing and optofluidics, greatly facilitating the advances in lab-on-chip technologies. Particularly, nano-structured photonic devices with high index contrast provide unique modal power distribution on a chip and enhanced optical gradient force. However, typically, particle trapping is studied in a static case, like calculating optical force and trapping stiffness at specific positions. There has been little report on nanoparticle trapping dynamics, by tracking the trajectory of a particle carried by flowing fluid, although this would be critical to deepen our understanding on particle trapping and manipulation and to analyze device performance for sensing and biological applications.

In this work, by solving motion equation numerically, we simulate the dynamics of nanoparticle under the influence of optical force and water field. It is shown that a nanoparticle can go along different paths before it gets trapped, strongly depending on its initial position relative to the integrated waveguide. Due to high index of the silicon waveguide, optical field enhancement produces complex optical force distribution, creating multiple traps on both top surface and sidewalls of the waveguide. It is found that the optical forces originating from different parts of an optical mode compete with each other in particle transport, creating a critical area where particle transport becomes unstable and causing dramatically different trapping locations. Additionally, undesirable trapping positions may reduce the sensitivity or even generate misleading results of a sensor, which makes dynamic analysis indispensable in the future.

10061-27, Session 6

Silicon-on-Insulator based Microsystem design for efficient in-vivo diagnostic and treatment

Muhammad Mujeeb-U-Rahman, Univ. of the Punjab (Pakistan); Axel Scherer, California Institute of Technology (United States)

Microscale optical devices enabled by wireless power harvesting and telemetry will facilitate manipulation and testing of localized environments (e.g. neural recording and stimulation, targeted delivery to cancer cells). Design of integrated optical microsystems will enable complex in-vivo applications, thus minimizing the requirement of tight optical focusing within the tissue or use of nanoparticles for such applications. Silicon-on-Insulator (SOI) based platforms provide a very powerful design tool as these can be used to fabricate both optoelectronic and microelectronic devices for miniaturized implantable systems. Near Infrared (NIR) biomedical optics dictates that optical power harvesting can outperform other methods (e.g. RF and acoustic) used for such miniaturized systems.

In this paper, we present design and integration techniques of such optical structures (e.g. optoelectronic photovoltaic systems) in microelectronics based SOI technologies. The design of optical elements in our system takes advantage of NIR therapeutic region and special device processing to have optically transparent window for backside illumination while using the front side CMOS design as efficient mirror structure to increase the efficiency of otherwise barely usable SOI devices. The results are based upon device testing for illumination through actual biological tissue. Numerical simulation results are also discussed to provide insights into thermal safety during system operation.

The design principles presented in this work can also be utilized for designing different optical components including optical sensors (based upon ratio of tissue and device absorption). The system presented in this work provides a unique platform for biomedical operations at microscale using optoelectronics powered by optical illumination.

10061-44, Session PSun

Liquid-phase reduction synthesis of mono-dispersed gold nanoparticles on glass microfluidic device with flow rate control

Yu Tanabe, Hiromasa Yagyu, Kanto Gakuin Univ. (Japan)

Gold nanoparticles (GNPs) in aqueous dispersion were synthesized on a low cost glass microfluidic device developed by authors. The effect of a channel width and a flow rate on the size distribution of synthesized GNPs was reported for synthesis mono-dispersed GNPs. Soda-lime glass substrates were processed by the micropowder blasting. Three holes were processed on upper substrate, and the Y-shaped microchannel was processed on bottom substrate. Tetrachloroauric (III) acid aqueous solution for Au ion and the mixture of aqueous solution of sodium citrate acid and tannic acid for reduction and the protection agent were injected to a microchannel in the device by syringe pump. From the analysis of absorption peak at around 530nm in absorption spectra, the synthesized GNPs on the device has sharpen peak in comparison with that of GNPs synthesized on the beaker. Moreover, the spectra with low flow rate showed sharpened peak in comparison with high rate. In the channel width of 200?m, the full width at half maximum (FWHM) at the absorption peak were 79.2nm for 0.05mL/min and 92.9nm for 0.06mL/min. Conversely, FWHM in the channel width of 400?m showed almost constant value. From TEM images of the synthesized GNPs, it was confirmed that the mono-dispersed GNPs with the mean diameter of 11.5nm and coefficient of variation of 0.09 was synthesized at the flow rate of 0.05mL/min and the channel width of 200?m. These results confirmed that low flow rate and small channel width were attributed to realize mono-dispersed GNPs.

10061-45, Session PSun

Laser generated superhydrophobic rose leaf surfaces for high adhesion droplet arrays

Sandra Stroj, Matthias Domke, FH Vorarlberg (Austria); Victor V. Matylitsky, Spectra-Physics Rankweil (Austria); Volha Matylitskaya, Stephan Kasemann, FH Vorarlberg (Austria)

Microfluidic systems are important tools in biology and life sciences in general. One important issue is the compatibility of the microfluidics device with the biological system that is usually sensitive to its environment. The control of chemical and biochemical reactions with minor influence by its boundary is important for numerous applications. Using microfluidic systems that operate in “open space” is one trend where a one-dimensional “smart” contact surface has to substitute the function of a three-dimensional device. We demonstrate a fabrication process for generation of rose leaf surfaces where superhydrophobic behaviour is combined with an extreme adherence of water droplets on the surface – the so called “Rose petal effect”.

The wetting behaviour of a surface is a complex problem where both, material properties and the surface topography influences the interaction of a liquid with the substrate surface. The patent-pending fabrication process (ClearSurfaceTM from Spectra-Physics®) is a combination of thin film structuring with a femtosecond laser followed by a wet oxidation step.

The sample is based on a quartz wafer where a layer of a-Si was deposited by sputtering. The a-Si layer was selectively structured by a femtosecond laser. After laser structuring the sample was thermally (wet) oxidized leading to a full conversion of the a-Si layer to quartz.

The final surface shows superhydrophobic behaviour with values of contact angle of up to 163° together with an extreme hysteresis value of up to 151° leading to an extreme adhesion of water droplets on the surface.

10061-46, Session PSun

High-sensitivity interpretation of lateral flow immunoassay results using lock-in thermography

Manu Pallapa, Ashkan Ojaghi, Nima Tabatabaei, Pouya Rezai, York Univ. (Canada)

Existing optical immunoassay readers based on image acquisition and processing algorithms rely on reflective signals (color intensity) of the surface of the lateral flow immunoassay (LFA) to interpret result. Although this method provides quantitative results, a large amount of signal from gold nanoparticles (GNP) trapped inside of the bulk of the LFA is lost, leading to unsatisfactory detection threshold and sensitivity. In this work, we report on a lock-in thermography detection system (excitation: 808nm; detection: 8-14μm) for detecting GNPs within the bulk of the LFA using the thermal-wave science principles. LFA samples in the range of 20mIU-0.2mIU were tested via lock-in thermography, human visual interpretation, and a commercial optical strip reader. While visual interpretation showed acceptable positive test confidence for higher concentrations (20mIU-8mIU), the confidence of true positive fell to 30% for 4mIU, 20% for 2mIU and 0% for lower concentrations. The optical strip reader showed an acceptable true positive confidence only up to 4mIU. However, thermal-contrast images obtained through lock-in thermography were able to identify GNPs located in the bulk at concentrations as low as 1mIU, confirming superior detection threshold and sensitivity of lock-in thermography over conventional methods. Miniaturization of this technique will enable a highly quantitative, low-cost, truly point-of-care total analysis test interpretation system for LFAs facilitating critical early stage detection of diseases or conditions.

10061-47, Session PSun

Numerical study of insulator-based dielectrophoresis method for circulating tumor cell separation

Mohammad Aghaamoo, Univ. of California, Irvine (United States); Arian Aghilinejad, Xiaolin Chen, Washington State Univ. Vancouver (United States)

Insulator-based dielectrophoresis (iDEP) is known as a powerful technique for separation and manipulation of bioparticles. In recent years, iDEP designs using arrays of insulating posts have shown promising results towards reaching high-efficient bioparticles manipulation. However, there is still an essential need for providing comprehensive design guidelines and further optimizing such devices. In this research, we utilized numerical simulation to study, in detail, insulating posts iDEP technique with the specific application of bioparticles separation. To achieve this, we first developed a robust numerical model to predict the electric and fluid flow fields' distribution, and how bioparticles are being manipulated inside the system. This enabled us to study the fundamental principles of such an iDEP method. In the next step, different design aspects of insulating posts iDEP were investigated. Specifically, we focused on the effect of posts geometry and configuration on the systems' key operation criteria such as the effectiveness of the electric field non-uniformity, the flow velocity distribution, the pressure drop inside the system, and shear stress rates. Furthermore, we studied how different electrodes' setup may affect the electric field distribution and consequently the microfluidic device performance. Finally, the developed numerical tool was used to demonstrate separation of circulating tumor cells (CTCs) from white blood cells (WBCs). For this purpose, MDA-231 breast cancer cells and Granulocytes were chosen as an indicator of CTCs and WBCs, respectively. Our developed numerical model and presented results lay the groundwork for design and fabrication of high-efficient insulating posts iDEP microchips.

10061-48, Session PSun

Numerical study of insulator-based dielectrophoresis method for circulating tumor cell separation

Mohammad Aghaamoo, Univ. of California Irvine (United States); Arian Aghilinejad, Xiaolin Chen, Washington State Univ. Vancouver (United States)

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10061-29, Session 7

Piezoelectric micromachined ultrasonic transducers and micropumps: from design to optomicrofluidic applications

Shadi Khazaaleh, Numan Saeed, Raquel Flores, Inas Taha, Mateusz T. Madzik, Jaime Viegas, Masdar Institute of Science & Technology (United Arab Emirates)

In this work, we present the experimental results of a new wafer-level production platform for aluminum nitride based piezoelectric micromachined ultrasonic transducers (PMUTs), operated by lower than 10 V peak-to-peak signals, and covering ultrasonic frequency ranges from 200 kHz up to 15 MHz, with measured axial displacements ranging from a few nanometers up to 1000 nm. The fabricated devices have a low footprint of (130x130) ?m2. The experimental results are in excellent agreement with finite-element method simulations. Micropump and micromixer designs based on such piezoelectric MEMS are reported. The integration of multiproject-wafer CMOS compatible pMUT chips with microfluidic devices is also discussed with potential applications in cell lysis and biosample preparation. Further applications of PMUTs not limited to microfluidic integration are also discussed, namely their application in ultrasonic gesture recognition for medical equipment interaction and in ultrasonography/ photo-acoustic imaging. Such integration promises compact, multimodal solutions for integrated biomedical systems.

10061-30, Session 7

Optimized AC electrothermal micromixing design for biofluid systems

Alinaghi Salari, Univ. of Calgary (Canada); Maryam Navi, Ryerson Univ. (Canada); Colin Dalton, Univ. of Calgary (Canada)

Electrokinetic fluid delivery techniques have many applications in biofluid transport systems. Among those, the electrothermal fluid transport technique is a highly effective method for fluids with high conductivities, in the order of 0.02-1 S/m. The ACET phenomenon has been mainly reported in the literature for micropumping and micromixing applications using coplanar asymmetric electrode arrays at the bottom of a microchannel. Recently, a novel ACET micropump based on a multi-electrode array system was reported. In this micropump, multiple asymmetric electrode arrays located on different sidewalls of the microchannel were utilized. Following this work, we implemented the same concept for a micromixing mechanism. For the sake of simplicity, only two coplanar microelectrode pairs, on the top and bottom of a 2D micro chamber, were considered. By applying different species concentration at one corner of the chamber, mixing of the fluid can be characterized throughout the chamber area. Simulations were performed using COMSOL Multiphysics. The results showed that using opposed asymmetric microelectrode pairs can provide a 74% decrease in the mixing time compared to identical pairs. Also, a chamber, which has two electrode pairs, can have a 67% decrease in mixing time compared to one which has only one pair.

10061-31, Session 7

CFD study of microfluidic oscillator characteristics for flow-separation control

Tawfiq Chekifi, Univ. Tahri Mohammed Béchar (Algeria)

The fluidic oscillator is an interesting device developed for passive flow measurement, flow separation and flow control applications. These patented microstructures can produce a high oscillating jet frequency with high flow velocity. Moreover, fluidic oscillators have no moving parts. Commercial CFD code FLUENT was used to perform analysis of flows in microfluidic oscillator, Numerical simulations were carried out for different flow conditions, where water and air were used as working fluids. The oscillation frequencies were identified by the discrete fast Fourier transform method (FFT). Furthermore a low-pressure vortex of fluid flow in the oscillating chamber was observed between the jet stream and the attachment wall. The effect of the operating pressure and the oscillating chamber shape on the microfluidic oscillator performance is investigated. The velocity fluctuations of the feedback flows through the two feedback channels and the output were determined quantitatively. In addition, the behaviour of the low-pressure vortex in both models is analyzed. Comparison of our numerical simulations with available previous work showed reasonably and good agreement, which demonstrate the accuracy of our models.

10061-32, Session 8

Mechano-optical plasmonic nanoantenna *(Invited Paper)*

Somin Eunice Lee, Univ. of Michigan (United States)

We present a mechano-optical nano-antenna capable of nanometer spatial resolution and stability over a broad temperature range. We theoretically demonstrate a matching condition for mechanical properties that is essential for maximizing thermal expansion differences across a broad temperature range. Mechano-optical nano-antennas should allow for spatiotemporal temperature mapping in future applications where precise measurement of local temperature is needed.

10061-33, Session 8

High efficiency isolation of cells in laminar flow microfluidic channels: breaking the mass transport limit

Xiangchao Zhu, Evan Peterson, Ahmet A. Yanik, Univ. of California, Santa Cruz (United States)

Biomarker-based isolation of circulating cells on functionalized surfaces in microfluidic devices is the basis of many modern biomedical diagnostics technologies. However, under typical laminar flow conditions, random diffusion of cells to the microfluidic channel walls is weak, a fundamental concern in microfluidics. We invented a novel technique to separate lateral fluidic drag forces and vertical mass transport to the channel walls and achieved high efficiency isolation of cells on microfluidic channel surfaces. We achieve to entangle lateral flow microfluidics from mass transport to the microfluidic channels and demonstrated highly specific and efficient capturing of target cells in laminar flow conditions.

10061-34, Session 8

Microfluidic separation of particles from whole blood using shear induced diffusion

Jian Zhou, Zhejiang Univ. (China) and Univ. of Cincinnati (United States); Chunglong Tu, Yitao Liang, Bobo Huang, Zhejiang Univ. (China); Ian Papautsky, Univ. of Cincinnati (United States); Xuesong Ye, Zhejiang Univ. (China) and Univ. of Cincinnati (United States)

Extraction of cells of interest directly from human whole blood is in high demand. However, it is extremely challenging due to the excessive cell populations leading to non-Newtonian hemodynamics. Herein, we describe a simple microfluidic approach to take the advantage of the concentrated cells for direct isolation of larger particles spiked in whole blood, which could shift the current paradigm in cell separation from low volume fraction sample to concentrated suspension and from the Newtonian to non-Newtonian fluid. Our device fabricated in Polydimethylsiloxane(PDMS) via standard soft photolithography consists of three inputs and three outputs. With saline solution flowing in the channel center and splitting the blood sample into two side streams, the spiked larger particles rapidly migrate from the whole blood to the saline stream without external force fields. Our experimental results has suggested such intriguing particle translocation is mainly attributed to the shear induced diffusion in concentrated suspensions as well as the viscoelastic property of blood. Inertial force can also contribute to this process. The harmonic interactions among these passive force fields have successfully led to extraction, focusing and separation of 18.7 μm –diameter particles from whole blood with a high efficiency (~89%) and a superb throughput (>10^6 cells per second), which outperforms existing approaches such as spiral microchannels. In summary, we have demonstrated a novel simple and effective separation scheme which directly works for complex bodily samples without a hassle of sample preparation. Our approach is very promising as it holds potential to separate cells from whole blood.

10061-35, Session 8

A novel micro-fluidic approach to produce controllable gas-in-liquid-in-liquid double emulsions

Cong Huang, Hongbo Zhang, East China Univ. of Science and Technology (China); Ruixue Yin, East China Univ. of Science and Technology (China); Katharina K. C. Koch, Fach Hochschule Lübeck (Germany); Wenjun Zhang, Univ. of Saskatchewan (Canada) and East China Univ. of Science and Technology (China)

We present a simple but robust way to produce monodispersed gas-in-oil-in-water (G/O/W) double emulsions with a novel non-planar microfluidic device. The non-planar design avoids complicated surface modification in device. By manipulating the flow rate of each phase, the structure of G/O/W microbubbles can be tuned precisely. The approach is also validated in generating G/W/O double emulsions.

Introduction

Gas-in-liquid-in-liquid (G/L/L) double emulsion bubble is an ideal contrast medium in contrast-enhanced ultrasonography. In recent years, several approaches have been developed for producing G/L/L microbubbles. However, these approaches are limited in several aspects, e.g., unstable quality control and need of complicated modification. These limitations may be overcome by a novel design of micro-fluidic devices and control of flows.

Experimental

A PDMS based non-planar device was designed and fabricated by multilayer photolithography.

The device is composed of an upstream junction and a downstream junction with the heights of 25 and 75 ?m respectively.

Experimental results proved that the device is capable in generating both G/O/W and G/W/O double emulsions. The value of coefficient variation of diameters is less than 1.4%, indicating high monodispersity. In addition, by tuning the flow rates of each phase, precise control over diameter, shell thickness and core number of G/L/L structure could be achieved.

Conclusion

In this article, an efficient approach to generate G/L/L double emulsions is described. The non-planar design of microfluidic device simplifies the fabrication process and provides reliable control for generation of both G/O/W and G/W/O microbubbles. Such microbubbles can be used in contrast-enhanced ultrasonography.

10061-36, Session 9

Multiplexed measurement of protein/peptide interactions using spectrally encoded beads *(Invited Paper)*

Huy Q. Nguyen, Kara K. Brower, Scott Longwell, Jagoree Roy, Stanford Univ. (United States); Bjorn Harink, Brian Baxter, Joseph L. DeRisi, Univ. of California, San Francisco (United States); Martha Cyert, Stanford Univ. (United States); Kurt S. Thorn, Univ. of California, San Francisco (United States); Polly Fordyce, Stanford Univ. (United States)

Multiplexed bioassays, in which multiple analytes are probed and tracked in a single experiment, have become increasingly important tools for basic research and clinical diagnosis. Spectrally encoded microparticles show great promise for these assays: by uniquely associating each analyte with a particular spectral code, analytes can be tracked throughout the course of an experiment via imaging alone. We recently developed a microfluidic platform capable of producing microparticles containing distinct ratios of lanthanide nanophosphors, each of which comprises a unique spectral code (MRBLEs, or Microspheres with Ratiometric Barcode Lanthanide Encoding), and demonstrated the ability to produce and distinguish > 1,100 distinct codes with high confidence. Here, we establish the utility of these MRBLEs for multiplexed bioassays by using them to probe how calcineurin (CN), a Ca2+/calmodulin-dependent phosphatase critical for the human immune response, recognizes its target substrates. We synthesize a library of 96 peptides containing single-site substitutions of two known CN substrates directly on the beads and show that the embedded codes are unaffected by the harsh chemicals required for solid-phase peptide synthesis. We then use these bead-bound peptide libraries to measure concentration-dependent binding behavior for CN interacting with up to 100 peptides in parallel and demonstrate semi-quantitative measurement of affinities for even weak protein peptide-interactions (~10-50 μM). We anticipate that this new platform will have broad utility for profiling a wide range of protein-peptide interactions, from understanding cellular signaling networks to profiling immune responses.

10061-37, Session 9

Microfluidic system for in-vitro hypoxia assays

Mathias Busek, Fraunhofer IWS Dresden (Germany); Stefan Grünzner, Fraunhofer IWS Dresden (Germany) and TU Dresden (Germany); Tobias Steege, Florian Schmieder, Udo Klotzbach, Frank Sonntag, Fraunhofer IWS Dresden (Germany)

For cell cultivation the oxygen supply is a key factor influencing cell vitality. An oxygen restriction is called hypoxia and induces several changes in cell metabolism, influencing for example tumor growth. Those hypoxia assays mostly use animal models like genetically modified mice or static human cell culture models. Results based on animal testing are hardly transferable to human medicine. Static human cell culture systems on the other hand mostly don´t consider cell-cell interactions. Besides Perfused, microfluidic 3D cell cultivation systems may be a new powerful tool to observe the impact of hypoxia.

Hereby presented is a microfluidic system, including a micro pump, an oxygenator and a cell culture chamber for perfusion controlled hypoxia assays. It consists of laser-structured polycarbonate (PC) foils and an elastomeric membrane which were joined together using thermal diffusion bonding. The high gas permeability of the elastomer ensures sufficient oxygen exchange at the included oxygenator. The oxygen content is measured with optical lifetime detection. This oxygen sensor, the micro pump, a controlling device and the gas mixture at the oxygenator forms a regulatory circuit to adjust the oxygen content in the cell culture chamber and helps to produce well-defined hypoxic conditions for the cells.

The working principle of the developed microfluidic system is demonstrated by applying different process gas mixtures to the oxygenator and observing the oxygen change in the cell culture chamber as well as the impact of hypoxia to cultivated cells with the help of typical hypoxia inducible factors (HIF).

10061-38, Session 9

Novel lab-on-a-chip platform for high throughput drug discovery with DNA-encoded chemical libraries

Stefan Grünzner, Fraunhofer IWS Dresden (Germany) and TU Dresden (Germany); Francesco V. Reddavide, TU Dresden (Germany); Christian Steinfelder, Mathias Busek, Udo Klotzbach, Frank Sonntag, Fraunhofer IWS Dresden (Germany); Yixin Zhang, TU Dresden (Germany)

The fast development of DNA-encoded chemical libraries (DECL) in the past 10 years has received great attention from pharmaceutical industries. It applies the selection approach for small molecular drug discovery. Because of the limited choices of DNA-compatible chemical reactions, most DNA-encoded chemical libraries have a narrow structural diversity and low synthetic yield. There is also a poor correlation between the ranking of compounds resulted from analyzing the sequencing data and the affinity measured through biochemical assays. By combining DECL with dynamical chemical library, the resulting DNA-encoded dynamic library (EDCCL) explores the thermodynamic equilibrium of reversible reactions as well as the advantages of DNA encoded compounds for manipulation/detection, thus leads to enhanced signal-to-noise ratio of the selection process and higher library quality. However, the library dynamics are caused by the weak interactions between the DNA strands, which also result in relatively low affinity of the bidentate interaction, as compared to a stable DNA duplex. To take advantage of both stably assembled dual-pharmacophore libraries and EDCCLs, we extended the concept of EDCCLs to heat-induced EDCCLs (hi-EDCCLs), in which the heat-induced recombination process of stable DNA duplexes and affinity capture are carried out separately. To over-come the

extremely laborious and repetitive manual process, a fully automated device will revolutionize the use of DECL in drug discovery.

Herein we describe a novel lab-on-a-chip platform for high throughput drug discovery with hi-EDCCL. A microfluidic system with integrated actuation was designed which is able to provide a continuous circulation by reducing the volume to a minimum. It consists of a cooled and a heated chamber for constant circulating. The system is capable to generate stable temperature above 75 °C in the heated chamber for melting the double strands and to cool the second chamber to less than 15 °C, for reannealing the reshuffling library. In the binding chamber (the cooled chamber) specific retaining structures are integrated. These hold back beads, which are modified with target protein, while the chamber is permanently flushed with library molecules. Afterwards the whole system can be flushed with buffer to wash out unspecific bound molecules. Then the protein-loaded beads with attached molecules can be eluted for further investigation.

10061-39, Session 9

Thermally-assisted acoustophoresis as a new stiffness-based separation method

Ata Dolatmoradi, Bilal El-Zahab, Florida International Univ. (United States)

The use of acoustophoretic separation devices provides a feasible means in biomedical diagnostics for label-free separation of diseased cells. Separation via acoustophoresis, however, has been restricted mainly to size contrast. This paper reports on a newly-developed acoustophoretic-based approach integrating ultrasonic and thermal actuators on the same platform to enable the separation of biological materials according to their mechanical properties. Using this method, we demonstrate the possibility of separating cell-mimicking liposomes based on their membrane stiffness. In a temperature-tuned microchannel with an overlaid ultrasonic standing wave, the acoustic contrast factor of a liposome is mainly determined according to its compressibility compared to that of medium. The sign of this factor was observed to flip to a negative value at a specific temperature, unique to the composition of the liposome. We hypothesize this sign switch is due to the thermotropic phase transitions in the liposome's membrane upon which an apparent effect on the compressibility is experienced by the liposome. The existence of a temperature window within which liposomes of different compositions were mechanically distinct enough to become differentiable in the acoustic radiation field enabled us to efficiently separate them with target outlet purities over 95%.

10061-40, Session 10

Organs-on-Chips approaches for complex disease models *(Invited Paper)*

Collin Edington, Linda G. Griffith, Massachusetts Institute of Technology (United States)

"Mice are not little people" – a refrain becoming louder as the strengths and weaknesses of animal models of human disease become more apparent. At the same time, three emerging approaches are headed toward integration: powerful systems biology analysis of cell-cell and intracellular signaling networks in patient-derived samples; 3D tissue engineered models of human organ systems, often made from stem cells; and micro-fluidic and meso-fluidic devices that enable living systems to be sustained, perturbed and analyzed for weeks in culture. This talk will highlight the integration of these rapidly moving fields to understand difficult clinical problems, with an emphasis on translating academic discoveries into practical use. In particular, technical challenges in modeling complex diseases with "organs on chips" approaches include the need for relatively large tissue masses and organ-organ cross talk to capture systemic effects. These constraints drive development of new strategies for perfusing organ models, as well as "mesofluidic" pumping and circulation in platforms connecting several organ systems, to achieve the appropriate physiological relevance.

10061-41, Session 10

Novel 3-dimensional gold micro-electrodes allow high resolution neural network recording

Pierre J. J. Wijdenes, Ryden Armstrong, Cezar Gavrilovici, Univ. of Calgary (Canada); Jong M. Rho M.D., Alberta Children's Hospital (Canada); Naweed I. Syed, Colin Dalton, Univ. of Calgary (Canada)

Introduction:

While micro-electrode arrays (MEAs) have offered new opportunities to better understand neural network formation and possible dysfunction, their potential is limited by the quality of the signal-to-noise ratio detected during neural tissue recordings. We present here a new BioMEMS design of a multi-electrode array with 3-dimensional micro-electrodes that can perform in-vitro recordings of neuronal network activity in intact mammalian brain slices. The recorded neural activity was then compared with previously reported results that were obtained with existing 3-dimensional and planar micro-electrodes and were found to be significantly better.

Fabrication and experimental method:

A multi-electrode array with 3-dimensional gold micro-electrodes was fabricated using a custom micro-fabrication process coupled with standard photolithography. All electrodes have a controlled height ranging from 50μm to 400μm, with their bases and shafts insulated, allowing for direct contact with neural cells only via the tips. Mice hippocampal brain slices with thicknesses ranging from 350μm to 500μm were interfaced with the micro-electrodes in-vitro and spontaneous neural activity (bursting and individual spikes) was recorded at multiple electrode sites.

Results and Discussion:

Neural activity from mammalian tissue was recorded up to 3.2mV, with the average noise reduced to 20μV. The maximum reported signal in the current literature is 1.5mV, with the noise at 40-60μV. This represents a significantly higher signal-to-noise ratio than most commercially available medical microsystems in this area. This approach offers new opportunities to record neural network phenomena with higher resolution and, for example, now allows the collection and analysis of drug screening data more accurately.

10061-42, Session 10

Tumor-on-a-chip: a new ally against cancer

Karolina Papera Valente, Mohsen Akbari, Afzal Suleman, Univ. of Victoria (Canada)

The uncontrolled cancer cell growth that originates a tumor alters the environment of a normal tissue, increasing interstitial pressure and creating hypoxic regions. The disorganized structure of cancer cells within a tissue can interfere with the efficient distribution of cytotoxic drugs, making it challenging to treat the tumor. This stressful situation can be mimicked using microfluidics. Microfluidic devices are cost-effective platforms that can be used to study cancer since they allow spatial and temporal control of the system. In this work, a breast tumor-on-a-chip was developed to study the impact of tumor size in the efficacy of cancer treatment. The chip comprises a central chamber and two lateral channels. The central chamber contains MCF-7 cells encapsulated in a 3D hydrogel matrix, in order to mimic the extracellular matrix environment. The lateral channels function as blood vessels where cytotoxic drugs and nutrients are injected and delivered to the cancer cells. Diffusive transport of molecules was ensured by posts that connect the central chamber to the lateral channels. Therapeutic effect of cytotoxic drugs was assessed by monitoring the drug concentration gradient profile inside the chips with central chambers of different dimensions and by evaluating cell viability using fluorescent dyes. Theoretical drug diffusion in the microfluidic device was simulated using Transport of Diluted Species module in COMSOL.

10061-43, Session 10

Microfluidic devices for stem-cell cultivation, differentiation and toxicity testing

Holger Becker, Thomas E. Hansen-Hagge, microfluidic ChipShop GmbH (Germany); Andreas Kurtz, Charité Universitätsmedizin Berlin (Germany); Ralf Mrowka, Universitätsklinikum Jena (Germany); Stefan Wölfl, Heidelberg Univ. (Germany); Claudia Gärtner, microfluidic ChipShop GmbH (Germany)

The development of new drugs is time-consuming, extremely expensive and often promising drug candidates fail in late stages of the development process due to the lack of suitable tools to either predict toxicological effects or to test drug candidates in physiologically relevant environments prior to clinical tests. We therefore try to develop diagnostic multi-organ microfluidic chips based on patient specific induced pluripotent stem cell (iPS) technology to explore liver dependent toxic effects of drugs on individual human tissues such as liver or kidney cells. Based initially on standardized microfluidic modules for cell culture, we have developed integrated microfluidic devices which contain different chambers for cell/tissue cultivation as well as on-chip cell-culture medium actuation with an on-board peristaltic pump and gas exchange using integrated membranes. The devices are manufactured using injection molding of thermoplastic polymers such as polystyrene or cyclo-olefin polymer. In the project, suitable surface modification methods of the used materials as well as hybrid integration of different elements had to be explored. We have been able to successfully demonstrate the seeding, cultivation and further differentiation of modified iPS, as shown by the use of differentiation markers, thus providing a suitable platform for toxicity testing and potential tissue-tissue interactions.

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10062-1, Session 1

Towards a comprehensive understanding of nonlinear energy deposition into transparent tissues *(Invited Paper)*

Alfred Vogel, Norbert Linz, Sebastian Freidank, Univ. zu Lübeck (Germany); Xiao-Xuan Liang, Univ. zu Lübeck (Germany) and Xi'an Jiaotong Univ. (China)

Plasma-mediated energy deposition into transparent tissues by tightly focused laser pulses involves a multitude of nonlinear interactions. They include multiphoton ionization, inverse Bremsstrahlung absorption, impact ionization, recombination, thermal ionization (T > 3000 K), radiative energy transfer via soft X-ray photons (T >> 10000 K), and thermomechanical processes.

We present a comprehensive picture of the interplay of these nonlinear interactions in a large parameter space ranging from femtosecond to nanosecond durations, and from UV to IR wavelengths. We cover energy densities from the level of nonlinear microscopy to values associated with strong shock waves and cavitation. Plasma structure and size are explored photographically, transmission measurements and plasma volume yield the plasma energy density, and the cavitation bubble size is used to measure the conversion of deposited energy into mechanical effects.

We find that precisely tunable energy deposition and nanoeffects are possible not only with ultrashort laser pulses but also with single-longitudinal-mode ns pulses of UV and VIS wavelengths. The effect size increases continuously for ultrashort pulses but exhibits a stepwise transition from nanoeffects to brightly luminescent plasma for longer pulse durations. Only when multiphoton-generation of seed electrons is a critical hurdle, breakdown sets in abruptly. In luminescent ns plasmas, electron-density-dependent avalanche ionization produces hot strings emitting soft x-rays. That homogenizes and inflates the plasma and limits the average energy density to about 40 kJcm-3, similar to fs breakdown. Well above threshold, the cavitation bubble energy is proportional to the pulse energy for all pulse durations and wavelengths. Consequences of our findings are illustrated on applications in cell and tissue surgery.

10062-2, Session 1

Evidence of femtosecond-laser pulse induced cell membrane nanosurgery

Nir Katchinskiy, Roseline Godbout, Abdulhakem Y. Elezzabi, Univ. of Alberta (Canada)

The presentation provides insight into the mechanism of femtosecond laser nanosurgical attachment of cells. In order to verify the hypothesis that hemifusion is the physical process that takes place during femtosecond laser-induced cell attachment, transmission electron microscope images of the cell membranes of retinoblastoma cells were taken. It is demonstrated that during the attachment of two cells using sub-10 femtosecond laser pulses, with 800 nm central wavelength, the phospholipid molecules of both cells hemifuse and form one shared phospholipid bilayer, at the attachment location. The two cell membranes coalesce and form one single membrane shared by both cells. Hemifusion of the membranes occurs due to laser-induced ionization process that led to an ultrafast reversible destabilization of the phospholipid layers of the cellular membranes, which resulted in cross-linking of the phospholipid molecules in each membrane. The attachment between the cells takes place across a large surface area, which results in strong physical attachment between the cells. The femtosecond laser pulse hemifusion technique can potentially provide a platform for precise molecular manipulation of cellular membranes, and for tissue engineering. Manipulation of the cellular membrane is an important procedure that could aid in studying diseases such as cancer.

10062-3, Session 1

In-vitro photo-translocation of antiretroviral drug delivery into TZMbl cells

Rudzani Malabi, Council for Scientific and Industrial Research (South Africa) and Univ. of South Africa (South Africa); Sello L. Manoto, Saturnin Ombinda-Lemboumba, Council for Scientific and Industrial Research (South Africa); Malik Maaza, Univ. of South Africa (South Africa); Patience T. Mthunzi-Kufa, Council for Scientific and Industrial Research (South Africa) and Univ. of South Africa (South Africa)

The current human immunodeficiency virus (HIV-1) treatment regime possesses the ability to diminish the viral capacity to unnoticeable levels; however complete eradication of the virus cannot be achieved while latent HIV-1 reservoirs go unchallenged. Therapeutic targeting of HIV-1 therefore requires further investigation and current therapies need modification in order to address HIV-1 eradication. This deflects research towards investigating potential novel antiretroviral drug delivery systems. The use of femtosecond (fs) laser pulses in promoting targeted optical drug delivery of antiretroviral drugs (ARVs) into TZMbl cells revolves around using ultrafast laser pulses that have high peak powers, which precisely disrupt the cell plasma membrane in order to allow immediate transportation and expression of exogenous material into the live mammalian cells. A photo-translocation optical setup was built and validated by characterisation of the accurate parameters such as wavelength (800 nm) and pulse duration (115 fs). Optimisation of drug translocation parameters were done by performing trypan blue translocation studies. Cellular responses were determined by cell morphology (using a light microscope), cell viability (Adenosine Triphosphate activity) and cell cytotoxicity (Lactate Dehydrogenase) assays were done to study the influence of the drugs on the cells. After laser irradiation high ARV drug cell viability was observed and high toxicity levels were observed after prolonged exposure of the cells. With minimal damage and high therapeutic levels of ARVs, fs laser assisted drug delivery system is efficient with benefits of non-invasive and non-toxic treatment to the cells.

10062-4, Session 1

Targeted femtosecond laser driven drug delivery within HIV-1 infected cells: In-vitro studies

Charles Maphanga, Council for Scientific and Industrial Research (South Africa) and Univ. of South Africa (South Africa); Saturnin Ombinda-Lemboumba, Sello L. Manoto, Council for Scientific and Industrial Research (South Africa); Malik Maaza, Univ. of South Africa (South Africa); Patience T. Mthunzi-Kufa, Council for Scientific and Industrial Research (South Africa) and Univ. of South Africa (South Africa)

Human immunodeficiency virus (HIV-1) still remains one amongst the world's most challenging infections since its discovery. Antiretroviral therapy is the recommended treatment of choice for HIV-1 infection taken orally. The highly active antiretroviral therapy (HAART) prevents the replication of HIV-1 and further destruction of the immune system, therefore enabling the body to fight opportunistic life-threatening infections, cancers, and also arrest HIV infection from advancing to AIDS. The major challenge with HAART is the inability to reach the viral reservoirs where the HIV-1 remains

latent and persistent, leading to inability to fully eradicate the virus. This study is aimed at initially designing and assembling a fully functional optical photo-translocation setup to optically deliver antiretroviral drugs into HIV-1 infected cells in a targeted manner using Gaussian beam mode femtosecond laser pulses In-vitro. The main objective of our study is to define the in-vitro drug photo-translocation parameters to allow future design of an efficient drug delivery device with potential in-vivo drug delivery applications. In our experiments, HEK 293T cells were used to produce HIV-1 enveloped pseudovirus to infect TZM-bl cells which were later treated with laser pulses emitted by a titanium sapphire laser (800nm, 1 KHz, 115fs, ~6.5μW) to create sub-microscopic pores on the cell membrane enabling influx of extracellular media. Changes in cellular responses were analysed using cell morphology studies, viability, proliferation, cytotoxicity, and luciferase assay. Controls included laser untreated cells incubated with the drug for 48 hours. The data in this study was statistically analysed using the SigmaPlot software version 11.

10062-5, Session 1

Photo-transfection and differentiation of mouse embryonic stem cells using femtosecond laser pulses

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Cellular manipulation by delivery of molecules into cells has been applied extensively in tissue engineering research. The different molecular delivery techniques range from viral and chemical agents to physical and electrical methods. Although successful in most studies, these techniques have been noted to have inherent side-affects that make experimental design a challenge. As of late, laser pulses have been used for drug and DNA delivery into cells via transient optical perforation of the cellular membrane. This non-invasive method causes no latent physical damage to mammalian cells treated and there is no risk of chemical interaction that might cause permanent genetic damage to the laser manipulated cell. In this study, we used femtosecond laser pulses to introduce transcription factors into embryonic stem cells. The aim was to cause differentiation of mouse embryonic stem cells into an endoderm layer, which facilitates early development of organ tissue in a developing embryo. Following transformation experiments, a transcription factor Sox17 was delivered into the cells via photo-poration using femtosecond laser pulses. Immunostaining of stem cells post laser irradiation showed that expression of fluorescent pluripotency markers decreased in cells containing the Sox 17 whilst the expression increased in the control experiment where no transcription factor was used. It was thus concluded that femtosecond laser pulses are capable of delivery of genetic material for cellular manipulation.

10062-6, Session 2

Investigation of the efficacy of ultrafast laser in large bowel excision

Syam Mohan P. C. Mohanan, Rainer J. Beck, Wojciech S. Gora, Heriot-Watt Univ. (United Kingdom); Sarah L. Perry, Mike Shires, David G. Jayne, Univ. of Leeds (United Kingdom); Duncan P. Hand, Jonathan D. Shephard, Heriot-Watt Univ. (United Kingdom)

Local resection of early stage tumors in the large bowel via colonoscopy has been a widely accepted surgical modality for colon cancer treatment. The conventional electrocautery techniques used for the resection of neoplasia in the mucosal or submucosal layer of colon tissue has been shown to create obvious thermal necrosis to adjacent healthy tissues and lacks accuracy in resection. Ultrafast picosecond (ps) laser ablation using wavelengths of 1030 and 515 nm is a promising surgical tool to overcome the limitations seen with conventional surgical techniques.

The purpose of this initial study is to analyze the depth of ablation and coagulation deployed by the laser as a function of pulse energy and fluence in an ex-vivo porcine model. Precise control of the depth of tissue removal is of paramount importance for bowel surgery where bowel perforation can lead to morbidity or mortality. Thus we investigate the regimes that are optimal for tissue resection and coagulation through plasma mediated ablation of healthy colon tissue. The ablated tissue samples were analyzed by standard histologic methods and a three dimensional optical profilometer technique. We demonstrate that ultrafast laser resection of colonic tissue can minimize the region of collateral thermal damage (<50 μm) with a controlled ablation depth. This surgical modality allows potentially faster and easier removal of early stage lesions and has the capability to provide more control to the surgeon in comparison with a mechanical or electrocautery device for excising critical organs such as the colon.

10062-7, Session 2

Supra-threshold epidermis injury from near-infrared laser radiation exposure prior to ablation onset

Michael P. DeLisi, Amanda M. Peterson, Engility Corp. (United States); Lily A. Lile, Air Force Research Lab. (United States); Gary D. Noojin, Aurora D. Shingledecker, David J. Stolarski, Engility Corp. (United States); Justin J Zohner, Engility (United States); Semih S Kumru, Air Force Research Laboratory (United States); Robert J. Thomas, Air Force Research Lab. (United States)

With continued advancement of solid-state laser technology, high-energy lasers operating in the near-infrared (NIR) band are being applied in an increasing number of manufacturing techniques and medical treatments. Safety-related investigations of potentially harmful laser interaction with skin are commonplace, consisting of establishing the maximum permissible exposure (MPE) thresholds under various conditions, often utilizing the minimally-visible lesion (MVL) metric as an indication of damage. Likewise, characterization of ablation onset and velocity is of interest for therapeutic and surgical use, and concerns exceptionally high irradiance levels. However, skin injury response between these two exposure ranges is not well understood. This study utilized a 1070-nm Yb-doped, diode-pumped fiber laser to explore the response of excised porcine skin tissue to high-energy exposures within the supra-threshold injury region without inducing ablation. Concurrent high-speed videography was employed to assess the effect on the epidermis, with a dichotomous response determination given for three progressive damage event categories: observable permanent distortion on the surface, formation of an epidermal bubble due to bounded intra-cutaneous water vaporization, and rupture of said bubble during laser exposure. ED50 values were calculated for these categories under various pulse configurations and beam diameters, and logistic regression models predicted injury events with approximately 90% accuracy. The distinction of skin response into categories of increasing degrees of damage expands the current understanding of high-energy laser safety while also underlining the unique biophysical effects during induced water phase change in tissue. These observations could prove useful in augmenting biothermomechanical models of laser exposure in the supra-threshold region.

10062-8, Session 2

Direct numerical simulation of microcavitation processes in different bio environments

Kevin Ly, Sy-Bor Wen, Texas A&M Univ. (United States); Morgan S. Schmidt, Robert J. Thomas, Air Force Research Lab. (United States)

Laser-induced microcavitation refers to the rapid formation and expansion of a vapor bubble inside the bio-tissue when it is exposed to intense, pulsed laser energy. With the associated microscale dissection occurring within the tissue, laser-induced microcavitation is a common approach for high precision bio-surgeries. For example, laser-induced microcavitation is used for laser in-situ keratomileusis (LASIK) to precisely reshape the midstromal corneal tissue through excimer laser beam.

Multiple efforts over the last several years have observed unique characteristics of microcavitions in biotissues. For example, it was found that the threshold energy for microcavitation can be significantly reduced when the size of the biostructure is increased. Also, it was found that the dynamics of microcavitation are significantly affected by the elastic modules of the biotissue. To explain these unique behaviors occurring during the rapid, physically complicated microcavitation process, direct numerical simulation that is based on first principles is required.

In this study, a direct numerical simulation of the microcavitation process based on equation of state of the biotissue is established. With direct numerical simulation, we are able to reproduce the dynamics of microcavitation in polyacrylamide (PAA) gel possessing different water concentrations. Also, we are able to predict the threshold laser energy of microcavitation for polystyrene microspheres in water. In addition to computationally reproducing the experimental results, the direct numerical simulation can serve as a tool to provide back calculations for exposed biotissue physical properties using experimentally measured dynamics of microcavitation of tissues under different laser/background conditions.

10062-9, Session 2

All-fiber laser at 1.94 μm: effect on soft tissue

Atasi Pal, Debasis Pal, Sourav Das Chowdhury, Ranjan Sen, Central Glass and Ceramic Research Institute (India)

The ability of laser to cut, coagulate, vaporise and ablate tissues through thermal effect leads to precision surgery. Thulium-doped fiber lasers (TFL) can emit at wavelength of strong water absorption near 1.94 μm and is thus currently being studied as a potential and possibly superior alternative to the Ho:YAG laser for soft tissue surgery. While laser wavelength is the primary parameter, laser power and waveform is also important to reduce the risk of unacceptable damage to the tissue. Rigorous investigation is essential to fix the laser parameter depending on the thermal properties of biological tissues before a treatment is offered. A fiber Bragg grating-based, all-fiber, continuous-wave as well as modulated, cladding pumped, thulium-doped fiber laser at 1.94 μm has been configured to deliver up to 10 W of laser power under pumping at 793 nm having efficiency of 32 %. The designed laser has been exposed to freshly sacrificed chicken breast with various laser power, exposure time and mode of operation; the formalin-fixed samples were evaluated by microscopy to study the affected tissue. The analysis reflects that, beside ablation, the laser power vaporises all water molecule and the adjacent tissues are blackened because of the carbon atom release, called carbonation. Surrounding the carbonation part there is necrosis region because of denaturation of proteins and collagen that leads to coagulation of tissue at high temperature. It has been observed that average width of carbonised region is 0.4 mm while coagulated region width is 1.3 mm having less dependence on the laser power up to 10 W. Increase of laser power increases the ablation depth with a reduction in the ablation diameter.

10062-10, Session 3

Pressure generation during neural stimulation with infrared radiation *(Invited Paper)*

Claus-Peter Richter, Northwestern Univ. (United States); Nan Xia, Chongqing Univ. (China); Xiaodong Tan, Yingyue Xu, Northwestern Univ. (United States)

Neural stimulation with infrared radiation (INS) has been used to stimulate peripheral and cranial nerves and the cortex. Following the absorption of the photon, its energy is converted into heat. Several mechanisms by which the heat is then converted into an action potential have been described. Temporally and spatially confined heating depolarizes the cell by changing the membrane capacitance, the activation of heat sensitive transient receptor potential (TRP) cation channels, and changes in intracellular calcium homeostasis. Furthermore a more general effect on ion channels has been discussed. Spatially and temporally confined heating, which occurs during INS also results in stress relaxation waves. The dispute is whether the resulting pressure is the dominating effect in cochlear INS. With this paper we will briefly review each of the mechanisms and will provide results from laser evoked pressure waves in small confined volumes. Custom fabricated pressure probes were used to determine the pressure in front of the optical fiber in a small dish and patch pipettes to measure corresponding temperature changes. Heating was spatially confined. The heat relaxation time was 35 ms. At 164 μJ/pulse, the pressure was estimated to be 114 dB (re 1 μPa). Pressure waves are generated during infrared laser stimulation. The contribution of a mechanical event still remains an open question regarding the mechanism for INS.

10062-11, Session 3

Short pulse laser induced thermo-elastic deformation imaging

Tianshi Wang, Erasmus MC (Netherlands); Tom Pfeiffer, Institut für Biomedizinische Optik, Univ. zu Lübeck (Germany); Min Wu, Erasmus MC (Netherlands); Wolfgang Wieser, Ludwig-Maximilians-Univ. München (Germany); Antonius F. W. van der Steen, Erasmus MC (Netherlands); Robert A. Huber, Institut für Biomedizinische Optik, Univ. zu Lübeck (Germany); Gijs van Soest, Erasmus MC (Netherlands)

Thermal-elastic displacement is related to the optical, mechanical and thermal properties of the tissue. In this study, we used 5ns duration short pulse laser to induce the thermal-elastic displacement by running the laser at 500 nm and 1200 nm wavelengths. The power of the laser is 2 mJ per pulse. The typical thermal-elastic displacement is sub micron order. In order to detect such tiny displacement, we used a super fast optical coherence tomography (OCT) system that is based on a 1.5 MHz Fourier Domain Mode Locked laser. The tiny displacement was detected as the phase shift between adjacent Alines in the OCT data. We conducted the ex vivo experiments in lipid and healthy arteries. The results show that 500 nm pulse laser can induce thermal-elastic displacement in healthy arteries. No significant displacement was detected in arteries with 1200 nm laser. However, 1200 nm can induce thermal-elastic displacement in lipid, and no significant displacement was detected with 500 nm wavelengths. The results show that thermal-elastic displacement detection can potentially be used for tissue characterization especially lipid detection for intravascular application.

10062-12, Session 3

Short infrared laser pulses increase cell membrane fluidity

Alexandra J. Walsh, Air Force Research Lab. (United States); Jody Cantu, General Dynamics Information Technology (United States); Bennett L. Ibey, Hope T. Beier, Air Force Research Lab. (United States)

Short infrared laser pulses induce a variety of effects in cells and tissues, including neural stimulation and inhibition. However, the mechanism behind these physiological effects is poorly understood. It is known that the fast thermal gradient induced by the infrared light is necessary for the biological effects. Therefore, this study tests the hypothesis that the fast thermal gradient induced in a cell by infrared light exposure causes a change in the membrane fluidity. To test this hypothesis, we used the membrane fluidity dye, di-4-ANEPPDHQ, to investigate membrane fluidity changes following infrared light exposure. Di-4-ANEPPDHQ fluorescence was imaged on a wide-field fluorescence imaging system with dual channel emission detection. The dual channel imaging allowed imaging of emitted fluorescence at wavelengths longer and shorter than 647 nm for ratiometric assessment and computation of a membrane generalized polarization (GP) value. Results in CHO cells show increased membrane fluidity with infrared light pulse exposure and this increased fluidity scales with infrared irradiance. Full recovery of pre-infrared exposure membrane fluidity was observed. Cholesterol depletion of CHO cell membranes also resulted in lower membrane fluidities and increased infrared effects. Finally, NG108 and primary neurons also exhibited increased membrane fluidity following infrared exposure. Altogether, these results demonstrate that infrared light induces a thermal gradient in cells that changes membrane fluidity.

10062-13, Session 3

Thermal confinement effects on the cell cytoskeleton due to infrared light exposure

John Mason, Texas A&M Univ. (United States) and Air Force Research Lab. (United States); Alexandra J. Walsh, National Research Council (United States) and Air Force Research Lab. (United States); Joel N. Bixler, Caleb C. Roth, Air Force Research Lab. (United States); Ronald A. Barnes, Air Force Research Lab. (United States) and National Research Council (United States); Jonathan V. Thompson, Texas A&M Univ. (United States) and Air Force Research Lab. (United States); Bennett L. Ibey, Hope T. Beier, Air Force Research Lab. (United States)

Infrared laser pulses with widths of a few milliseconds have been shown to stimulate and block action potentials in neurons. While the precise stimulation or inhibition mechanism is unknown, evidence indicates the response of the neuron originates from the rapid thermal gradient produced by the absorption of the infrared light by the surrounding water. The absorption is high enough and the pulse sufficiently short to achieve thermal confinement, which may generate a photomechanical effect or pressure wave felt by the target tissue. Here, we investigate the impact of this thermal confinement on the cell cytoskeleton. Using confocal microscopy, we observe the impact of the infrared laser pulses on Chinese Hamster Ovarian (CHO-K1) cells and primary hippocampal neurons expressing fluorescent proteins tagged to actin. Pulse energies are varied to explore the effects of radiant exposures that result in neural stimulation, as well as action potential block. By monitoring the distortion and/or disruption of actin filaments, we gain insight into possible effects of thermal confinement on biological cells, and the secondary mechanical effects (thermal expansion) that may result from exposure to a thermal gradient, as well as possible intracellular effects that may initiate a mechanical response in the cell.

10062-14, Session 3

Antivascular effect induced by photo-mediated ultrasound

Xinmai Yang, The Univ. of Kansas (United States); Zizhong Hu, Univ. of Michigan (United States); Haonan Zhang, Tongji Univ. (China); Aghapi G. Mordovanakis, Yannis M. Paulus, Xueding Wang, Univ. of Michigan (United States)

We developed a novel localized antivascular method, namely photo-mediated ultrasound therapy (PUT), by applying synchronized laser and ultrasound pulses. PUT relies on high optical contrast among biological tissues. Taking advantage of the high optical absorption of hemoglobin, PUT can selectively target microvessels without causing unwanted damages to the surrounding tissue. Moreover, PUT working at different optical wavelengths can selectively treat veins or arteries by utilizing the contrast in the optical spectra between deoxy- and oxy-hemoglobin. Through our experiments and theoretical simulations, we demonstrated that cavitation might have played a key role in PUT. The addition of a laser pulse to an existing ultrasound field can significantly improve the likelihood of inertial cavitation, which can induce microvessel damage through its mechanical effect. In comparison with conventional laser therapies, such as photothermolysis and photocoagulation, the laser energy level needed in PUT is significantly lower. When a nanosecond laser was used, our in vivo experiments showed that the needed laser fluence was in the range of 4 to 40 mJ/cm^2. Histology findings confirmed that fibrin clots were developed in the microvessels in the treated region, while no damage was found in the surrounding tissue, clearly indicating that the treatment effect of PUT was limited perfectly in the microvessels.

10062-15, Session 4

Spectral domain optical coherence tomography (SD-OCT) of laser-induced skin damage thresholds at 1070 nm

Adam Boretsky, Michael P. DeLisi, Engility Corp. (United States); Morgan S. Schmidt, Semih S. Kumru, Air Force Research Lab. (United States); Aurora D. Shingledecker, Gary D. Noojin, Amanda M. Peterson, David J. Stolarski, Engility Corp. (United States); Benjamin A. Rockwell, Robert J. Thomas, Air Force Research Lab. (United States)

The near-infrared (NIR) window is an increasingly popular region of the electromagnetic spectrum for biomedical, industrial, military, and communications applications. NIR lasers are often used in the biomedical field due to the relatively high depth of penetration in tissue. This property is well suited for low-powered imaging applications but high-powered NIR laser exposure to the skin may cause subsurface defects that are not readily apparent using conventional detection methods. Traditional threshold studies rely on visible outcomes at the tissue surface but do not account for any subsurface effects. Maximum permissible exposure (MPE) limits found in laser safety standards are largely based on experimental data derived from minimum visible lesion (MVL) studies or theoretical modeling.

This study was designed to incorporate advanced imaging and visualization techniques into the traditional evaluation of laser injury thresholds. A series of studies using 1070-nm lasers were used to identify the MVL skin damage thresholds for the Yucatan miniature pig model with a 1-cm beam diameter operating under both continuous wave (CW) and pulsed conditions. The depth-resolved SD-OCT images were acquired at one and 24 hours after laser exposure. Multiple time points were chosen to evaluate the acute impact of the laser radiation and the persistent effects following the initial thermal response. Tissue biopsies were collected at corresponding laser exposure sites. Morphological changes observed in the SD-OCT images associated with sub-threshold, threshold, and supra-threshold laser damage were compared with the assessments of a certified pathologist based on H&E stained tissue sections.

The use of depth-resolved imaging techniques, such as SD-OCT, may help supplement future laser injury threshold studies to better understand the biological effects of exposure to highly penetrating laser sources operating within the NIR window.

10062-16, Session 4

Depth-resolved photothermal tissue alteration measurement by phase-resolved OCT

Shuichi Makita, Yoshiaki Yasuno, Univ. of Tsukuba (Japan)

Photothermal effect causes tissue alterations due to heat generated by light absorption.

Thermo-elastic and thermo-refractive changes occur as heat is increased.

Proteins will be unfolded when temperature over threshold.

It is difficult to predict them inside tissue because of their complex mechanisms.

The distribution of the alterations depend on homogeneity of tissue properties and illuminated light conditions.

In this study, we perform local optical path length (LOPL) measurement during laser illumination.

Phase-resolved optical coherence tomography (OCT) can detect the depth-resolved small OPL inside tissues.

For measurement, 1-um spectral-domain OCT was used.

OCT M-mode scan was applied during 532-nm laser illumination to ex vivo porcine eyes.

The LOPL change measurement reveals instantaneous alterations of retinal tissue.

In some cases, large LOPL change was observed at retinal pigment epithelium (RPE) which is pigment rich tissue.

After certain duration of laser illumination, LOPL change suddenly increase.

It was appeared at RPE or other retinal tissues above RPE.

These are probably indicates the starting point of denaturation.

The difference of location of large LOPL appeared may depend on the existence of blood above the RPE.

In conclusion, LOPL change measurement based-on phase-resolved OCT technique may be promising to predict and investigate the applications of photothermal heating.

10062-17, Session 4

Correlating measured transient temperature rises with damage rate processes in cultured cells

Michael L. Denton, Air Force Research Lab. (United States)

Two disparate approaches were used to study thermal damage rate processes in cultured retinal pigment epithelial cells. Laser exposure parameters included 2-μm laser exposure of non-pigmented cells and 532-nm exposures of cells pigmented at a variety of melanosome particle densities. Photothermal experiments used a thermal camera to record temperature histories, while fluorescence microscopy of the cell monolayers identified threshold damage at the boundary between live and dead cells. Photothermal exposure durations ranged from 0.05-20 s, and the effects of varying ambient temperature were investigated. Temperature during heat transfer using a water-jacketed cuvette was recorded with a fast microthermister, while damage and viability of the suspended cells were determined as percentages. Exposure durations for the heat transfer experiments ranged from 40-70 s. Empirically-determined kinetic parameters for the two heating methods were compared with each other and with values found in the literature. Results were also discussed in terms of thermodynamics and the compensation law.

10062-18, Session 4

Laser driven short-term thermal angioplasty: enhancement of drug delivery performance by heating with tension

Kao Suganuma, Rie Homma, Keio Univ. (Japan); Natsumi Shimazaki, Keio Leading-edge Lab. of Science and Technology (Japan); Emiyu Ogawa, Tsunenori Arai, Keio Univ. (Japan)

To enhance drug delivery performance of drug eluting balloon (DEB) against re-stenosis, we have proposed a heating drug delivery during balloon dilatation using our laser driven short-therm thermal angioplasty which realize to suppress surrounding thermal injury. We studied an influence of vessel dilatation parameters on the heating drug delivery. These parameters were classified into two different forces, that is, circumferential tension and inter-luminal pressure. We think these parameters were not able to determine only by balloon pressure.

The circumferential tension with 0-30 mN/mm^2 was added to a carotid artery using an automatic stage. Various temperature solutions with 37, 60 and 70? of hydrophobic fluorescent Rhodamine B with 3 ?g/ml in concentration were put on pig carotid wall. We measured a defined drug delivery amount as well as delivery depth by a microscopic fluorescence measurement on the cross section of the solution delivered vessel.

In the case of 37?, we found the intima surface drug amount with 7 mN/mm^2 was increased as 10-30 times as other tension values. On the other hand, at 60, 70?, we found the optimum tension with 30 mN/mm^2. We found the drug delivery enhancement might be related to the change of super microscopic surface structure of the vessel. We predict that the collagen thermal denaturation of the vessel wall might play important role to the drug delivery.

10062-19, Session 4

Modeling of the thermal response of human skin

Oleg O. Myakinin, Samara Univ. (Russian Federation); Ilya V. Krasnikov, Amur State Univ. (Russian Federation); Alexander Doronin, Yale Univ. (United States); Alexey Y. Seteikin, Amur State Univ. (Russian Federation); Valery P. Zakharov, Samara Univ. (Russian Federation); Igor Meglinski, Univ. of Oulu (Finland)

In this work, we present a novel method for simulation of the thermal response of human skin to laser irradiation. The approach is based on the mixed solution of Monte Carlo modeling with heat transfer equation calculations taking into account considering air convention and blood flow. Laser irradiation of biological tissue is widely used in medical treatment applications e.g. Photodynamic Threapy (PDT). In the developed model yields solution in two steps: 1. Monte Carlo simulation of the distribution of absorbed light energy in biological tissue; 2. Calculation of the amount of released heat by solving the heat-transfer equation using finite elements methodology by utilizing Penne's equation of bioheat as the core of the mathematical model for biological tissue temperature calculations. The final algorithm is implemented using Compute Unified Device Architecture (CUDA) framework designed for massive parallel computations on multiple NVidia Tesla K80 Graphics Processing Units (GPU) and online interactive application for simulation of heat transfer calculations has been developed. We present results of temperature distributions inside skin tissue as well as evaluation of radiation dozes for PDT application. Moreover, result of comparative analysis between our algorithm and methods developed by several other groups is presented.

10062-37, Session PMon

Multiple scattering of polarized light in uniaxial turbid media with arbitrarily oriented linear birefringence

Soichi Otsuki, National Institute of Advanced Industrial Science and Technology (Japan)

Optical birefringence is expected to be a potential probe for examining disease progression or treatment response, However, the birefringence modifies many polarization effects in a complex manner, making difficult to optically analyze birefringent tissues. We have shown that the so called reduced effective scattering Mueller matrix completely and precisely represents the multiple scattering in turbid infinite plane media that is measured by illuminating the medium with a thin light beam [Otsuki, J. Opt. Soc. Am A 33, 988 (2016)]. In the present study, the reduced matrices were simulated for backward scattering from uniaxtial turbid media with arbitrarily oriented birefringence using a Monte Carlo method. The Lu-Chipman decomposition could factorize the matrices and afford the polarization parameters in two-dimension. When the birefringence axis is inclined both to the surface (x-y) plane and to the z axis, the azimuthal dependence of the scalar retardance around the illumination point shows a large offset, which suggests that photons behave quite differently under the birefringence according to their polarization state and that the circular polarizations are converted to the elliptical polarizations with different orientations depending on the propagation azimuth. This is in contrast to the case in which the birefringence axis is oriented parallel to the x-y plane [Otsuki, Appl. Opt. 55, 5652 (2016)]; photons propagating in the medium experience the retardance in nearly the same way, when they are polarized +-45 deg. linearly and circularly; the two polarizations are converted one into the other via the elliptical polarizations.

10062-38, Session PMon

Laser spectroscopic diagnosis of photodynamic therapy

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In this study we have diagnosis of the photodynamic therapy by using different optical methods utilizing visible light for characterization of tissue that have been developed and evaluated. The feasibility of many of these methods has been demonstrated in animal experiment. in vivo. fifteen albino mice were divided to Control group, tumor group and treated group which injected by 5,10,15,20-Tetra(4-methoxyphenyl)porphyrin (TMP) and irradiated with diode laser wavelength 650nm.

Fluorescence spectroscopy is an optical method that can provide information about detection early cancers or premalignant which is dependent on changes in the fluorophore concentration or distribution. The spectra of Flavins and Porphyrin were discussed, using the line excitation wavelength 450 nm,405 nm respectively, the fluorescence emission spectra (FES) were recorded in the region from 420-700nm. The tumor control shows a prominent peak at 605.5nm, 606 nm and 601 nm for treated and normal respectively, which due to porphyrin. The emission peak of flavin at 513nm, normal control and treated are much more intense than the tumor. Its vis versa of porphyrin. The reason for the decreased autofluorescence seem to be a decrease in the amount of oxidized forms of flavins.

Fourier Transform Infrared, Raman spectroscopy and photo acoustic imaging and spectroscopy. These techniques used for tissue characterization and detection of lipids and proteins. The spectra were recorded and analyzed to obtain the variations between different types of tissues has been used in the experiments.

10062-39, Session PMon

The underlying structure of skin wrinkles: a hyperspectral approach to crows feet

Germain Puccetti, Ashland Inc. (United States)

Visually perceived skin wrinkles are well known to possess an underlying structure not apparent on the surface of the skin. This underlying structure can be brought out by polarized hyperspectral imaging. Wrinkle patterns of eye crow's feet are used as example to show a deeper existent pattern and its characterization. The deeper structure can be observed in the near ultraviolet region of the spectrum, especially at 420 and 460nm which are well suited to image skin surface inhomogeneities and bilirubin within the medium and deep epidermis. Imaging in the 500nm range can serve as a larger scale topology reference because of its deeper penetration into the upper dermis. This serves to bring out the deeper wrinkle pattern as imprinted by collagen anisotropies around deep folds. The approach has potential applications in evaluating the seriousness of wrinkle patterns or the extent of subcutaneous acne scars and thereby the efficacy of treatments.

10062-40, Session PMon

Development of a novel Zebrafish model of stroke using photochemical thrombosis and femtosecond-laser ablation

I-Ju Lee, Ian Liau, National Chiao Tung Univ. (Taiwan)

The use of animal models is indispensable for the understanding the pathophysiology or improvement the therapeutic strategies of stroke; however, conventional rodent animal models of stroke require sophisticated surgery and lack of reproducibility. Herein we report a method of confocal imaging-guided laser-induced stroke in zebrafish in vivo. Through photochemical thrombosis or femtosecond laser ablation, we were able to control the position of thromboembolic blood clot or extravascular bleeding in the vasculature of living zebrafish. The mortality and hemodynamic outcome were evaluated for zebrafish after induced thrombosis or bleeding at selected cerebral blood vessels. To demonstrate a prospective application of our approach for the understanding the outcome of stroke on a living animal, we evaluate the brain damage in vivo and the swimming behavior after initiating ischemic stroke. We expect that our model may not only improve our understanding of the pathophysiology of stroke but also facilitate development of therapeutic interventions.

10062-41, Session PMon

Monte Carlo mathematical modelling of the interactions between light and skin tissue of newborns

Olga Kozyreva, Alexandra Pushkareva, ITMO Univ. (Russian Federation)

A model of interactions between light and skin tissue was reviewed. For the present study the skin of newborns was examined. The characteristics of newborns skin tissue were taken into account when modeling. In the developed model the skin was introduced in the three layers: the epidermis, the basal layer and the dermis. The thickness of the skin layers correspond with the structure of newborns skin. Absorbance of each layer in the visible and near infrared regions of the spectrum was determined by the absorption of the three main skin chromophores: blood, melanin and water. The formula of the scattering and absorption coefficients of blood is given in the study. This paper presents the study of the blood oxygenation effect on the signal of diffusely scattered radiation for the three distances between the source and the receiver of radiation: 0.3 mm, 0.6 mm and 1.5 mm. The calculation was obtained using Monte Carlo mathematical modeling.

A detailed description of the model is given. The adequacy of suggested model has been tested by comparing calculated characteristic with the experimental results obtained by means of double integral sphere. The results show that the wavelength range which provides sufficiently accurate measurements depends on the distance between the source and the receiver of radiation and the certain data is provided. For the distance of 0.3 mm this range is at 700-780 nm, 950-1000 nm; for 0.6 mm it is at 640-670 nm, 760-780 nm, and 850-870 nm; for 1.5 mm at 620-740 nm.

10062-42, Session PMon

In vivo monitoring laser tissue interaction using high resolution Fourier-domain optical coherence tomography

Hang Chan Jo, Dong Jun Shin, Dankook Univ. (Korea, Republic of); Jin-Chul Ahn, Phil-Sang Chung, Dae Yu Kim, Beckman Laser Institute Korea, Dankook Univ. (Korea, Republic of)

Laser-induced therapies include laser ablation to remove or cut target tissue by irradiating high-power focused laser beam as well as laser coagulation to clot damaged or leaky blood vessels. These laser treatments are widely used tools for a minimally invasive surgery and retinal surgical procedures in clinical settings. In this study, we demonstrate laser tissue interaction images of various sample tissues using high resolution Fourier-domain optical coherence tomography (Fd-OCT). We use a Q-switch diode-pumped Nd:YVO4 nanosecond laser (532nm and 1064nm wavelengths) with a 4W maximum output at a 20 kHz repetition rate to ablate ex vivo and in vivo samples including chicken breast and mouse ear tissues. The Fd-OCT system acquires time-series B-scan images at the same location during the tissue ablation experiments with 532nm and 1064nm laser irradiation. The real-time series of OCT cross-sectional (B-scan) images compare structural changes of 532nm and 1064nm laser ablation using same and different laser output powers. Laser tissue ablation efficiencies are measured by the width and the depth of the tissue ablation from the B-scan images as well as the area of the tissue ablation from the en-face volumetric images. In vivo B-scan images depict microvascular changes at the dermis region by blood coagulation using the two different laser irradiations (532nm and 1064nm). These experiments can demonstrate non-invasive quantification of the laser tissue ablation efficiency as well as qualitative assessment of blood coagulation

10062-43, Session PMon

Simulation and study of temperature caused by laser irradiated and photoacoustic signal distribution

Shulian Wu, Liangjun Hu, Fujian Normal Univ. (China)

Photoacoustic imaging which is based on the optical absorption properties of biological tissue has been becoming a hot research topic in recent years due to its advantages of scalability and novel contrast mechanisms. In this study, the link of the tissue temperature increased by laser irradiated and photoacoustic signal distribution has been established from experiment and simulation to further understand the effect of thermal-acoustic. Concretely, a finite element analysis computer simulation was developed to model the fragmentation response of tissue during irradiation of laser. Thermal caused by laser energy and acoustic mechanisms were considered for allowing the laser settings to be predicted during the course of treatment. Then, the experiment about the photoacoustic monitoring the temperature changes were displayed, and the results compare with the simulation results to reveal the relation of temperature changes with photoacoustic signal distribution.

10062-44, Session PMon

Modelling skin diffuse reflectance spectra in the near-infrared spectral range

Gatis Tunens, Inga Saknite, Janis Spigulis, Univ. of Latvia (Latvia)

Monte Carlo method was used in this study to simulate diffuse reflectance spectra of the human skin to better understand the optical properties of the human tissue in the near-infrared wavelength range. By comparing the simulation spectra to experimentally taken spectra, we can estimate the relative volume fractions of different chromophores that have distinct absorption spectra in this wavelength range (water, lipids, collagen and elastin).

A Matlab based graphical user interface was created for faster spectral analysis and spectra comparison with the ability to set different optical parameters of the tissue model for the simulation program input and read the output of Monte Carlo simulation programs like MCML - Monte Carlo for Multi-Layered media, created by Lihong Wang and Steven Jacques.

To understand how each optical property of the tissue effects of the diffuse reflectance spectra, different changes in the optical parameters (refractive indices, scattering coefficient, layer geometry etc.) of the simulation model were performed and the resulting spectra thoroughly examined. The information gathered was then used to create more accurate tissue models that mirror the properties of the experimentally measured tissue.

The results of this research show potential for non-invasive determination of different properties of the human tissue by an inverse Monte Carlo approach if some parameters of the tissue are already known.

10062-46, Session PMon

Pros and cons of characterising an optical translocation setup

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The delivery of genetic material and drugs into mammalian cells using femtosecond (fs) laser pulses is escalating rapidly. This novel light based technique achieved through a precise focusing of a laser beam on the plasma membrane is called photoporation. This technique is attained using ultrashort laser pulses to irradiate plasma membrane of mammalian cells, thus resulting in the accumulation of a vast amount of free electrons. These generated electrons react photo-chemically with the cell membrane, resulting in the generation of sub-microscopic pores on the cell membrane enabling a variety of extracellular media to diffuse into the cell. This study is aimed at critically analysing the “do’s and don’ts” of designing, assembling, and characterising an optical translocation setup using a femtosecond legend titanium sapphire regenerative amplifier pulsed laser (Gaussian beam, 800 nm, 1 KHz, 115 fs, and an output power of 850 mW). Main objective in our study is to determine optical photo-translocation parameters which are compatible to the plasma membrane. Such parameters included beam profiling, testing a range of laser fluencies, beam quality, and laser-cell interaction time. In our study, Chinese Hamster Ovary-K1 (CHO-K1) cells were photoporated in the presence of trypan blue or propidium iodide to determine optimal parameters for photo-poration experiment. An average power of approximately 6.5 μW, exposure time of 30 ms, with a laser diffraction limited spot of ~1.1 μm diameter at the focus worked optimally without any sign of cytoplasmic blebbing. Cellular responses post laser treatment was analysed using cell morphology studies, cell viability, and transfection efficiency quantified.

10062-47, Session PMon

The role of numerical aperture in efficient estimation of spatially resolved reflectance by a Monte Carlo light propagation model

Matic Ivancic, Peter Naglic, Franjo Pernu?, Bo?tjan Likar, Miran Bürmen, Univ. of Ljubljana (Slovenia)

Diffuse reflectance spectroscopy is a non-invasive technique that can be used to measure the optical properties of biological tissues. The optical properties are defined by the tissue chromophores and tissue morphology, and as such provide unique information that can be used to detect tissue abnormalities. Spatially resolved measurements of the backscattered light allow separation and extraction of the absorption and scattering properties of the sample. However, efficient extraction of the optical properties greatly relies on the appropriate use of the light propagation model. In this study, a GPU-accelerated Monte Carlo light propagation model for multi-layered tissues is investigated.

For a given number of launched photon packets, the signal-to-noise ratio of the computed spatially resolved reflectance significantly depends on the numerical aperture of the detection scheme. If realistic small numerical apertures are used, such as commonly encountered in optical fiber probes or in particular hyperspectral imaging systems, the simulation times required to obtain adequate signal-to-noise ratio of the reflectance can become very long, especially at longer source-detector separations and for complex probe tip geometries. In this paper, we investigate the influence of a virtually increased numerical aperture on the simulated spatially resolved reflectance and its potential for reducing the simulation time. The methodology is also evaluated by an inverse Monte Carlo model, where the influence of the virtually increased numerical aperture is quantified in terms of errors obtained for the estimated absorption and reduced scattering coefficients and the sub-diffusive phase function parameter ?.

10062-48, Session PMon

Preservation media analysis for in vitro measurements of endogenous UV fluorescence of liver fibrosis in bulk samples

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Chronic liver disease and cirrhosis (i.e. liver fibrosis) were the twelfth leading cause of death in 2010 in the U.S.A., and seem to continue increasing as mentioned in the 2013 CDC report. The gold standard of diagnosis for liver fibrosis continues to be biopsy sample and its corresponding histology assessment. However, histological evaluation has several shortcomings related to various factors, such as sampling, inter- and intra-observer variations, etc. Hence, a non-subjective, minimally-invasive, and quantifying technique may support development and evaluation of a fibrosis regression treatment. The build-up of elastin, proteoglycans, and different types of collagen in the extracellular matrix of liver fibrosis may result on changes of the endogenous fluorescence of tissue. We propose to use the UV spectroscopy for studying the endogenous fluorescence of molecules in the fibrotic tissue. In this work, we evaluate the fluorescence excitation/emission matrix in the UV range for several samples of murine hepatic tissue preserved in different media. We used a spectrofluorometer to get the UV fluorescence matrices in 4 mm3 tissue samples. The ultimate goal is to identify excitation/emission pair that characterizes the fibrosis accumulation in tissue for quantitation task. Chemical changes on tissue, by the formaldehyde preservation, alter the profile and the intensity of the autofluorescence spectra from the first minute until thirteenth week. To avoid these drawbacks, phosphate-buffered saline (PBS) or Dulbecco's Modified Eagle Medium (DMEM) were used to get more stable in vitro measurements. PBS buffer showed to be the suitable preservation medium to study the endogenous fluorescence of molecules in the fibrotic tissue.

10062-49, Session PMon

Dynamic behavior of microtubules following terahertz excitation

Jeremy M. Moix, James E. Parker III, General Dynamics Information Technology (United States); Ibtissam Echchgadda, Air Force Research Lab. (United States)

In this study, we present large scale molecular dynamics simulations of a solvated microtubule to examine vibrational energy absorption and dissipation mechanisms, and to describe the expected behavior of the microtubule following terahertz (THz) excitation. Results of the computed microtubule absorption spectra and vibrational density of states in the terahertz regime are presented, along with an analysis of the vibrational dephasing rates of the tubulin center of mass dynamics, which are shown to be overdamped.

10062-50, Session PMon

Terahertz Radiation Effect on DNA Methylation

Catherine Millar-Haskell, Brady McMicken, David Evans, Air Force Research Lab. (United States); Cesario Z. Cerna, General Dynamics Information Technology (United States); Ibtissam Echchgadda, Air Force Research Lab. (United States)

Recent reports (1-4) have shown that exposure to terahertz (THz) radiation can influence gene expression in various cell types. We hypothesize that THz-induced gene regulation may in part be caused by DNA methylation/demethylation. In this study, we explored the thermal and non-thermal effects of THz radiation on DNA methylation in neuronal cells. Cells were exposed to 2.52 THz radiation at high and low power intensities for different time durations, and changes in global DNA methylation were examined following each exposure.

10062-51, Session PMon

Increasing the quality and germination gymnosperms by photonics methods

Alexey Iakovlev, A. Durova, Saint Petersburg State Forest Technical Univ. (Russian Federation); Sergey V. Kascheev, Aleksandr S. Grishkanich, Andrey A. Mak, Julia Ruzankina, ITMO Univ. (Russian Federation)

Reforestation and forest management are among the high-priority issues nowadays. Active deforestation, urbanization, natural processes destroying forestlands make scientists search for the ways of safe, advanced, and rapid reforestation in different areas all over the world. Seeds of Spruce fir and Siberian larch were taken as a material for experimental procedures. The seeds were exposed to radiation of the following wavelengths: 405 nm 500 mW, 450 nm 3000 mW, 532 nm 550 mW, 640 nm 1000 mW. Every experiment was made on a group of 100 seeds 4 times. The results were compared with control group of 100 un-irradiated seeds. Germination report was made on 7th, 10th and 15th day. An analysis of the results of

experimental work carried out by the following data were obtained:

- For all Picea abies seed laser irradiation 450 mW 3000 nm with a duration of more than 240 marked increase in the percentage of germination. The best results are observed when irradiated in the range from 420-900 nm.

- The largest increase in the average length of the roots of seedlings Picea abies nabolyudaetsya using irradiation 3000 mW 450 nm laser.

- In research with seeds Larix sibirica best results in an increase in germination obtained by irradiating laser 532 nm 550 mW.

- The largest increase in average root length of seedlings Larix sibirica as in the case of Picea abies were observed using laser irradiation to 450 nm 3000 mW

In conclusion, we can note an ambiguity of results, complicated interdependence between exposure time, wavelength and seed reactions of different species.

10062-52, Session PMon

Accurate quantification of changes in tissue chromophore concentrations by investigating and minimizing effects of DPF: a computational study

Xinlong Wang, Hanli Liu, The Univ. of Texas at Arlington (United States)

Hemodynamic and metabolic responses to functional brain stimulationcan be measured by broadband near infrared spectroscopy (bb-NIRS), but their accuracy is hampered by inter-chromophore cross talk due to improper selection of differential pathlength factor (DPF). DPF is time-dependent (t) and wavelength-dependent (?);both temporal and spectral shapes crucially affect the accuracy of chromophore concentration determination. To select proper DPF(t, ?), various approximations have been utilized by researchers. However, without knowing ground truth of real concentration changes as a gold-standard reference, the question of which selections or algorithms of DPF(t, ?) would provide accurate quantification of tissue chromophore concentrations still remains unsolved. In this study, a computational simulation approach was performed. Initial baseline chromophore concentrations and selected values of concentration changes were predefined as gold standards, based on which respective theoretical attenuation (or optical density) spectra were generated with 2% noise added. Then, the simulated attenuation spectra were fitted for estimated chromophore concentration changes with several commonly-used algorithms of DPF(?, t). The fitting process for the chromophore concentration changes was based on the modified Beer-Lambert law and executed by using multi-linear regression with 161 wavelengths. The percentage of quantification/fitting errors caused by different DPF(?, t) approximations with respect to the initially predefined concentration changes are computed and compared. Approximate 50% underestimation of chromophore concentration changes were observed for all of the commonly used and/or simplified DPF(?, t) algorithms. Accordingly, a proper correction factor was introduced for compensation, and an optimal time-invariant, wavelength-dependent DPF(?) was selected that can minimize the cross talk.

10062-53, Session PMon

Difference among human normal, Barrett's dysplasia and adenocarcinoma revealed by autofluorescence spectroscopy

Jun Chen, Zhong Zhang, Tianjin Medical Univ. General Hospital (China)

Previous study of optical screening of cancer has shown that difference of native fluorescence spectra can be used to distinguish cancer tissue from normal tissue. The native fluorophores, such as NADH and FAD, are involved in the oxidation of fuel molecules, and therefore, direct monitoring of NADH fluorescence dynamically can interpret the metabolic activity of cells. Usually the metabolic rate of advanced metastatic cancer cells is greater than that of less advanced cancer cells, causing the effect known as hypoxia, which was found by Warburg.

The aim of the present research is to determine if the native fluorescence spectroscopy approach is effective to detect changes of fluorophore compositions among different types of human Esophagus tissues. Human Esophagus tissues, such as normal, Barrett's, Dysplasia and Adenocarcinoma, were excited by the selective excitation wavelength of 351 nm laser. The contributions of principle biochemical components to fluorescence spectra from the tissue samples were investigated using the different non-negative constraint blind source separation methods. The higher relative content of NADH and lower collagen contents were observed in the Adenocarcinoma in comparison with the normal and Dysplasia tissues. This work shows the changes of relative contents of collagen and NADH obtained using native fluorescence spectroscopy may present potential criteria - for detecting different types of human Esophagus tissues.

10062-20, Session 5

Photosensitization reaction induced hemolysis in a cuvette observed with hemoglobin absorption spectrum of various species

Risa Hamada, Emiyu Ogawa, Tsunenori Arai, Keio Univ. (Japan)

To reveal hemolysis phenomena induced by a photosensitization reaction and the reaction environment, we measured absorption spectrum of a blood sample to analyze hemoglobin oxidation and resolved oxygen desorption dynamics. The quartz glass cell with 1 mm optical path length was used as a cuvette. Erythrocytes suspension medium of hematocrit 0.625 with 30 μg/ml talaporfin sodium was used as a sample. A red diode laser of 664 nm wavelength was emitted to the cuvette with 120 mW/cm2 in irradiance for 0-40 J/cm2. Absorption spectra of the sample were obtained before and after the photosensitization reaction by a spectrophotometer. Multiple regression analysis was employed to obtain concentrations of various hemoglobin species from measured absorption spectrum. Comparing to 0 and 40 J/cm2, met hemoglobin and deoxygenated hemoglobin concentration increased 0.12 g/dL and 0.01 g/dL, respectively. Oxygenated hemoglobin concentration decreased 0.11 g/dL. A linear relation ship between met hemoglobin concentration change and the hemolysis progression was confirmed (R2=0.96). Oxygen environment could also be presented by oxygen pressure calculated from the concentration of oxygenated and deoxygenated hemoglobin. These obtained hemoglobin concentration changes may indicate hemolysis progress and oxygen environment. We think this simple optical measuring method could reveal both hemolysis and the reaction oxygen environment.

10062-21, Session 5

Trans-cranial infrared laser stimulation induces hemodynamic and metabolic response measured by broadband near infrared spectroscopy on human forehead

Xinlong Wang, Sahil Sunil Nalawade, Divya Dhandapani Reddy, Fenghua Tian, The Univ. of Texas at Arlington (United States); F. Gonzalez-Lima, The Univ. of Texas at Austin (United States); Hanli Liu, The Univ. of Texas at Arlington (United States)

Transcranial infrared laser stimulation (TILS) uses infrared light (lasers or LEDs) for nondestructive and non-thermal photobiomodulation on the human brain. Although TILS has shown its beneficial effects to a variety of neurological and psychological conditions, its physiological mechanism remains unknown. Cytochrome-c-oxidase (CCO), the last enzyme in the electron transportation chain, is proposed to be the primary photoacceptor of this infrared laser. In this study, we wish to validate this proposed mechanism. We applied 8 minutes in vivo TILS on the right forehead of 11 human participants with a 1064-nm laser. Broad-band near infrared spectroscopy (bb-NIRS) from 740-900nm was also employed near the TILS site to monitor hemodynamic and metabolic responses during the stimulation and 5-minute recovery period. For rigorous comparison, we also performed similar 8-min bb-NIR measurements under placebo conditions. A multi-linear regression analysis based on the modified Beer-Lambert law was performed to estimate concentration changes of oxy-hemoglobin (?[HbO]), deoxy-hemoglobin (?[Hb]), and cytochrome-c-oxidase (?[CCO]). We found that TILS induced significant increases of ?[CCO], ?[HbO] and a decrease of ?[Hb] with dose-dependent manner as compared with placebo treatments. Furthermore, strong linear relationships or interplays between ?[CCO] versus ?[HbO] and ?[CCO] versus ?[Hb] induced by TILS were observed in vivo for the first time. These relationships have clearly revealed close coupling/relationship between the hemodynamic oxygen supply and blood volume versus up-regulation of CCO induced by photobiomodulation. Our results demonstrate the tremendous potential of bb-NIRS as a non-invasive in vivo means to study photobiomodulation mechanisms and perform treatment evaluations of TILS.

10062-22, Session 5

Evaluation of electrical propagation delay with cardiomyocytes by photosensitization reaction in vitro

Marika Doi, Emiyu Ogawa, Tsunenori Arai, Keio Univ. (Japan)

In order to study cardiomyocyte electrical conduction damage by a photosensitization reaction (PR) mostly comes from outside of the cells in a few minutes after the PR, we studied propagation delay of contact action potential with cardiomyocyte by the PR. To determine appropriate PR condition for tachyarrhythmia ablation, a precise electrophysiological experiment in vitro has been preferable. We measured the contact action potential using a microelectrode array system of which information may be correct than conventional Ca ion measurement. We investigated the propagation delays of an evoked potential to evaluate the electrical conduction damage by the PR. Rat cardiomyocytes were cultivated for 5-7 days on a dish with which 64 electrodes were patterned, in an incubator controlled to 37?, 5% CO2. The following conditions were used for the PR: 30-40 ?g/ml talapordfin sodium and 290 mW/cm2, 0-40 J/cm2 for an irradiation. A 2D map was obtained to visualize the propagation delays of the evoked potential. The propagation speed, which was calculated based on the measured propagation delays, was decreased by about 50% on average of all electrodes after the PR. Therefore, we think 2D propagation delays measurement of the evoked potential with contact action potential measuring system might be available to evaluate the acute electrical conduction damage of cardiomyocyte by the PR.

10062-23, Session 5

Extracellular talaporfin sodium-induced photosensitization reaction with various albumin animal species on myocardial cells in vitro

Emiyu Ogawa, Keio Univ. Graduate School (Japan); Tsunenori Arai, Keio Univ. Graduate School (Japan) and Keio Univ. (Japan)

It is reported that the albumin has different structure among animal species. We have proposed a new methodology of cardiac ablation using talaporfin sodium-induced photosensitization reaction with short drug-light interval to realize immediate and permanent therapeutic effect by singlet oxygen production mainly in the interstitial space. The photosensitization reaction efficacy with different animal species should be investing to consider the optimal animal therapeutic model to evaluate the therapeutic effect of new cardiac ablation methodology. We studied the cell-killing efficacy of extracellular photosensitization reaction using talaporfin sodium on myocardial cells in vitro with different albumin animal species: human, canine, bovine, and porcine serum albumin. We obtained that the albumin concentration tendency on the binding ratio and cell lethality was different among the animal species but there was no correlation between binding ratio and cell lethality. We found that the cell lethality dependence on albumin concentration showed 2 different groups, human-canine and bovine-porcine. We think that the canine might be useful as a therapeutic animal model since the cytotoxicity tendency on albumin concentration was similar with that of human albumin. These cell lethality tendency difference would be suggested to explain by the existence of the diazepam site that talaporfin sodium binds mainly.

10062-24, Session 5

Mapping of electrophysiological response to transcranial infrared laser stimulation on the human brain in vivo measured by electroencephalography

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Transcranial infrared laser stimulation (TILS) is a non-destructive and non-thermal photobiomodulation therapy or process on the human brain; TILS uses infrared light from lasers or LEDs and has gained increased recognition for its beneficial effects on a variety of neurological and psychological conditions. While the mechanism of TILS has been assumed to stem from cytochrome-c-oxidase (CCO), which is the last enzyme in the electron transportation chain and is the primary photoacceptor, no literature is found to report electrophysiological response to TILS. In this study, a 64-channel electroencephalography (EEG) system was employed to monitor electrophysiological activities from 15 healthy human participants before, during and after TILS. A placebo experimental protocol was also applied for rigorous comparison. After recording a 3-minute baseline, we applied a 1064-nm laser with a power of 3.5W on the right forehead of each human participant for 8 minutes, followed by a 5-minute recovery period. In 64-channel EEG data analysis, we utilized several methods (root mean square, principal component analysis followed by independent component analysis, permutation conditional mutual information, and time-frequency wavelet analysis) to reveal differences in electrophysiological response to TILS between the stimulated versus placebo group. The analyzed results were further investigated using general linear model and paired t-test to reveal statistically meaningful responses induced by TILS. Moreover, this study will provide spatial mapping of human electrophysiological and possibly neural network responses to TILS for first time, indicating the

potential of EEG to be an effective method for monitoring neurological improvement induced by TILS.

10062-25, Session 6

Assessment of geometry in 2D immune systems using high accuracy laser-based bioprinting techniques

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The immune system is a very complex system that comprises a network of genetic and signaling pathways subtending a network of interacting cells. The location of the cells in a network, along with the gene products they interact with, rules the behavior of the immune system. Therefore, there is a great interest in understanding properly the role of a cell in such networks to increase our knowledge of the immune system response. In order to acquire a better understanding of these processes, cell printing with high spatial resolution emerges as one of the promising approaches to organize cells in two and three-dimensional patterns to enable the study the geometry influence in these interactions. In particular, laser assisted bio-printing techniques using sub-nanosecond laser sources have better characteristics for application in this field, mainly due to its higher spatial resolution, cell viability percentage and process automation. This work presents laser assisted bio-printing of antigen-presenting cells (APCs) in two-dimensional geometries, placing cellular components on a matrix previously generated on demand, permitting to test the molecular interactions between APCs and lymphocytes; as well as the generation of two-dimensional structures designed ad hoc in order to study the mechanisms of mobilization of immune system cells. The use of laser assisted bio-printing, along with APCs and lymphocytes emulate the structure of different niches of the immune system so that we can analyse functional requirement of these interaction.

10062-26, Session 6

Novel flexible polarimetric probe for enhanced cystoscopy

Sarah Forward, Univ. of Toronto (Canada) and Princess Margaret Cancer Ctr. (Canada); Martin Sidler, Karen J. Aitken, The Hospital for Sick Children (SickKids) (Canada); Adam Gribble, Univ. of Toronto (Canada); Darius J. Bagli, The Hospital for Sick Children (SickKids) (Canada); I. Alex Vitkin, Univ. of Toronto (Canada) and Princess Margaret Cancer Ctr. (Canada)

By 2018, 1 billion people worldwide will be affected by partial bladder outlet obstruction (PBOO). Most commonly caused by enlarged prostate in men, PBOO results in elevated urine volumes and pressures in the bladder. These lead to bladder wall morphology changes (i.e. changes in collagen fiber realignment) causing bladder dysfunction, kidney failure, and hypertrophy. Micro-remodeling and alignment of the smooth muscle lining the bladder is a good indicator of bladder function, thus quantifying this could be advantageous in the assessment and treatment of PBOO.

As is common in urology, surveillance and assessment of PBOO is typically conducted via white light cystoscopy where biopsies can be taken, but this is rather inaccurate and invasive. Furthermore, current treatment options such as bladder augmentation neglect underlying bladder architecture. Thus there is a need of a more accurate, precise, and minimally invasive alternative to white light cystoscopy with enhanced information content, to enable more informed and thus improved management and treatment of PBOO and related pathologies.

A promising method to non-invasively probe the biophysical properties of tissue is polarimetry which is an imaging technique that measures tissue properties up to 4 mm in depth using polarized light. For example, polarimetry reveals the presence of tissue asymmetry (perhaps linked to extracellular matrix (ECM) alignment), its optical activity (associated with concentration of chiral molecules such as glucose), and its depolarization (linked to micro-morphological differences between normal and pathologic tissues). Polarimetry thus offers a wealth of potential useful biomedical information in (bladder) oncology – for example, ECM has been shown to organise cellular behaviour, thus playing critical role in cancer pathogenesis and possibly its metastatic spread.

Previous work has shown that changes in bladder wall morphology can be accurately characterized with polarimetry, e.g. the polarimetry-derived metric of linear retardance visualizes regions of distending bladder obstruction, a major cause of bladder dysfunction. However, this work was done in ex vivo preclinical bladder disease models. Thus, the next logical translational step is to engineer a flexible polarimetric probe suitable for in vivo clinical deployment.

The difficulty in implementing a fiber-optic based flexible polarization probe is that the polarization state of light is not only affected by the tissue ("signal"), but is also inherently altered by the fiber probe itself ("noise"). I detail these issues and outline how we resolve them in my presentation.

The over-arching goal of my research is to develop and implement a refined in vivo tissue polarimetry technology for a rapid, minimally invasive information-rich assessment of urological pathology. The development of a new polarization-based methodology specifically targeted at costly, complex, and poorly managed urological diseases should offer previously unavailable tissue polarimetry metrics and provide a clear translational path for in vivo clinical deployment in the bladder, with additional exciting applications in mucosal characterization of other hollow organs.

10062-27, Session 6

In-vitro validation of photoplethysmography for a direct measurement of volume arterial elastic modulus

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Increased Arterial Stiffness (AS) is associated with the development of cardiovascular diseases (CVDs) and is a parameter used for the prediction of its development at an early stage. A number of indices derived from Photoplethysmography (PPG) and pressure signals are known for their use in the estimation of AS. We propose a novel surrogate method for a direct measurement of volume elastic modulus (Ev) using the stress-strain relationship for identification of AS and prediction of CVD progress before vascular lesions induced symptoms.

An in-vitro setup that mimics the human circulation in health (Model 2) and disease (Model 2) has been developed. The system allows control of different patterns of the hemodynamic forces on the wall/fluid interface. Photoplethysmography and pressure sensors were incorporated in the system (for both models) enabling the continuous real-time measurement of PPG and pressure signals. Utilising these signals the measurement of the elastic modulus, from the stress-strain curve, was possible. The method was evaluated against the gold standard mechanical testing technique (Instron measurement). The results showed strong agreement with the gold standard measurement with a percent error of 0.26% and 1.9% for Model 1 and Model 2, respectively.

To our knowledge, this approach is the first to propose a direct measurement of volume arterial elastic modulus using pressure and PPG signals. This work has proven the feasibility of such approach, and with emerging non-invasive pressure measurements; the method can be further evaluated in an in vivo setting. Also, this study sheds light on the

relation between PPGs with pulsatile flow, where arterial wall elasticity and hemodynamic forces are crucial key factors in the formation of the PPG signal.

10062-28, Session 6

Using laser induced breakdown spectroscopy and acoustic radiation force elasticity microscope to measure the spatial distribution of corneal elasticity

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Purpose: The nonhomogeneous structure of the cornea suggests a unique spatial distribution of corneal elasticity. Non-invasive measurement of this distribution is critical to understanding how biomechanics control corneal stability and refraction. We show that the anterior cornea is more rigid than the underlying posterior stromal bed without disturbing the overall corneal structure.

Methods: Corneas from fresh porcine eyes (Sierra Medical, Whittier, CA) were excised from the globe leaving a 2 mm scleral rim intact. The corneal samples were suspended in collagen gelatin (10% w/w) within a water tank filled with deionized, degassed water. The water tank was attached to a 3-D mechanical stage allowing for precise control of cavitation bubble placement within the cornea. Femtosecond laser pulses induced optical breakdown and produced cavitation in the anterior and posterior cornea. A confocal ultrasonic transducer applied 6.5 ms acoustic radiation force-chirp bursts to the bubble at 1.5 MHz while monitoring bubble position using pulse-echoes at 20 MHz. A cross-correlation method was used to calculate bubble displacements. Maximum bubble displacements are inversely proportional to the Young's modulus. The laser induced breakdown spectroscopy (LIBS) were measured in the anterior and posterior cornea to see whether the laser induced plasmas signals will show relationship to Young's modulus.

Results: Bubble displacement was 18-25% greater in the posterior cornea relative to the anterior cornea. This indicates a larger Young's modulus in the anterior cornea in the direction orthogonal to the corneal surface. But the laser induced breakdown spectroscopy gave the similar results for different locations of cornea.

Conclusions: Our results show that the anterior cornea is stiffer than the posterior cornea and that ARFEM is capable of non-invasively measuring the distribution of corneal elasticity while the LIBS does not have such ability.

10062-29, Session 6

Identification and quantification of neurotransmitter mixture by terahertz spectroscopy

Yan Peng, Univ. of Shanghai for Science and Technology (China)

Terahertz spectroscopy has been widely used for investigating the fingerprint spectrum of different substances. For cancerous tissues, the greatest difficulty is the absorption peaks of various substances contained in tissues overlap with each other, which are hard to identify and quantitative analyze. As a result, it is very hard to measure the presence of cancer cell and then to diagnose accurately. In this paper, we select five typical neurotransmitters (?-aminobutyric acid, L-glutamic acid, dopamine hydrochloride, inositol and creatine) to measure their THz spectra with different mixture ratios. By choosing characteristic absorption peaks and using the least square method, we can identify the ingredient and proportion of each substance with the accuracy up to 94%. These results provide important evidences for identifying cancer cells and obtaining exact quantitative analysis.

10062-30, Session 7

Physically based radiative transfer framework for hyperspectral modelling of light interaction with volumetrically inhomogeneous scattering tissue-like media

Alexander Doronin, Yale Univ. (United States); Alexander Bykov, Univ. of Oulu (Finland); Holly E. Rushmeier, Yale Univ. (United States); Igor Meglinski, Univ. of Oulu (Finland)

In the current report we present further developments of a unified Monte Carlo-based computational model and explore hyperspectral modelling of light interaction with volumetrically inhomogeneous scattering tissue-like media. The developed framework utilizes voxelized representation of the medium and considers spatial/volumetric variations in both structural e.g. surface roughness and wavelength-dependant optical properties. We present the detailed description of algorithms for modelling of light-medium interactions and schemes used for voxel-to-voxel photon packet transitions. The results of calculation of diffuse reflectance and Bidirectional Scattering-Surface Reflectance Distribution Function (BSSRDF) are presented. The results of simulations are compared with exact analytical solutions, phantom studies and measurements obtained by a low-cost experimental system developed in house for acquiring shape and subsurface scattering properties of objects by means of projection of temporal sequences of binary patterns. The computational solution is accelerated by the graphics processing units (GPUs) and compatible with most standard graphics/ and computer tomography file formats.

10062-31, Session 7

Analysis of nanoparticles optical propagation influence in biological tissue simulating phantoms

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The applications of nanoparticles in optical techniques of diagnosis and treatment of biological tissues are increasing. Image contrast can be improved in diagnostic approaches such as fluorescence, spectroscopy or optical coherence tomography. The therapeutic effect can be increased if nanoparticles are previously incorporated in the biological tissue. This is the case in thermotherapy, or in Photodynamic Therapy. All these applications take advantage of specific properties of the nanoparticles involved, either optical up- or down-conversion, thermal confinement or the ability to act as a drug-carrier.

Although many biomedical applications that involve nanoparticles are being proposed and tested, there is a need to take into account the influence of those nanoparticles on optical radiation propagation. The previously mentioned optical treatment and diagnosis techniques assume a particular optical propagation pattern, which is altered by the addition of nanoparticles. This change depends on the nanoparticle material, shape, size and concentration, among other parameters. In order to try to quantify these changes, in this work several phantoms that include different nanoparticles are built and measured, in order to try to estimate the influence of nanoparticles in optical propagation. A theoretical model of optical propagation, which takes into account the absorption and scattering changes in the medium, is also considered. Nanoparticles of different sizes from 40 nm to 1 μm are analyzed. Nanoparticle materials of interest in biomedical applications are employed, particularly TiO2, ZnO2 and Au. The results are relevant in diagnosis interpretation of images and treatment outcome evaluation when nanoparticles are present.

10062-32, Session 7

T-Opt: A 3D Monte Carlo simulation for light delivery design in photodynamic therapy

Norihiro Honda, Hisanao Hazama, Kunio Awazu, Osaka Univ. (Japan)

The interstitial photodynamic therapy (iPDT) with 5-aminolevulinic acid (5-ALA) is a safe and feasible treatment modality of malignant glioblastoma. In order to cover the tumour volume, the exact position of the light diffusers within the lesion is needed to decide precisely. The aim of this study is the development of evaluation method of treatment volume with 3D Monte Carlo simulation for iPDT using 5-ALA. Monte Carlo simulations of fluence rate were performed using the optical properties of the brain tissue infiltrated by tumor cells and normal tissue. 3-D Monte Carlo simulation was used to calculate the position of the light diffusers within the lesion and light transport. The fluence rate near the diffuser was maximum and decreased exponentially with distance. The simulation can calculate the amount of singlet oxygen generated by PDT. In order to increase the accuracy of simulation results, the parameter for simulation includes the quantum yield of singlet oxygen generation, the accumulated concentration of photosensitizer within tissue, fluence rate, molar extinction coefficient at the wavelength of excitation light. The simulation is useful for evaluation of treatment region of iPDT with 5-ALA.

10062-33, Session 7

Simulation analysis of the transparency of cornea and sclera

Chih-Yao Yang, National Taiwan Univ. (Taiwan)

Both consist of collagen fibrils, sclera is opaque whereas cornea is transparent for optical wavelengths. By employing the pseudospectral time-domain (PSTD) simulation technique, we model light impinging upon cornea and sclera, respectively. To analyze the scattering characteristics of light, the cornea and sclera are modeled by different sizes and arrangements of the non-absorbing collagen fibrils. Various factors are analyzed, including the wavelength of incident light, the thickness of the scattering media, position of the collagen fibrils, size distribution of the fibrils. Simulation results show that the transparency of the cornea, the opacity of sclera can be accounted for by the scattering effect of light.

10062-34, Session 8

Measuring the backward part of the phase function: a new setup design

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The scattering phase function (the probability distribution of the scattering angle) is intimately associated with the cellular organization and ultrastructure of tissue. Since these physical parameters change during e.g. carcinogenesis; quantification of the phase function and related parameters may allow for improved non-invasive, in vivo discrimination between healthy and diseased tissue. Furthermore, for the derivation of models to interpret measured optical signals, assumptions about the phase function of tissue are often made – regularly assuming a Modified Henyey Greenstein. However, in contrast to other optical properties, the phase function has not yet been extensively measured for different tissue types.

With conventional goniometers, the exact backscatter direction of 180 degrees cannot be measured. Especially for techniques that detect backscattered light – such as Optical Coherence Tomography and Elastic Scattering spectroscopy – the details of the backward part of the phase function will have a considerable impact on the measured signal.

We have therefore developed a setup that can measure the backward part of the phase function: 134 to 180 degrees. Our design is based on full field Optical Coherence Tomography. We detect all angles simultaneously with a camera, while scanning the reference mirror. The phase function scales with the amplitude of the OCT signal for each angle. We will show our results for validation measurements on two silica bead samples of 200 nm and 400 nm beads.

10062-35, Session 8

Study of the effect of temperature on the optical properties of latin skins

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Photodynamic therapy (PDT) is a very effective technique in treating certain types of cancer, among the most common, skin cancer. PDT requires the presence of three elements: the photosensitizer, light and oxygen; without the presence of any of these three, PDT does not work. Light the penetration depth into the tumor depends on both the characteristics of the tissue to be treated and the wavelength. As the light dose to be administered in each lesion depends on the optical properties of the tissue, all the effects that change these properties should be considered to choose suitable doses. There are some studies that determine what is the maximum dose of radiation tolerated for certain types of skin, but is still unknown what is the influence of the temperature on the optical properties, especially for darker skin types. In this study, we analyze the optical properties of skin in vivo of different Latin volunteers in order to study the influence of the temperature changes on the optical properties and thereby to define more precisely the dose of light to be received by each patient in a personalized way. The optical properties of skin in vivo, were investigated using an optical system that includes an integrating sphere, a tungsten lamp and a spectrophotometer. Using the experimental set-up it was possible to obtain spectra reflectance of various volunteers and from this measurement, the absorption coefficient was recovered by Inverse Adding Doubling (IAD) program.

10062-36, Session 8

Enhanced blood laser Doppler velocimetry using different materials of seeding particles

Ashraf F. El-Sherif, Military Technical College (Egypt)

Blood supply is necessary for the proper functioning of all body organs as blood carries all the nutrients and oxygen that our body needs to stay healthy. Various diseases cause an impaired supply of blood to the organs. The measurement of the blood flow can therefore provide essential information for the diagnosis of diseases like Atherosclerosis. Since changes in blood flow occurs with early stage of disease detection, a fast, reliable and non-invasive blood flow measurement technique called Laser Doppler Velocimetry (LDV) is used.

This paper optimizes the measurement on optical properties of human blood seeded with different materials, the choice of seeding particles materials and sizes which are the most important parameters that will increase the signal to noise ratios and improves the performance of the LDV.

The study shows relative change of the optical properties (μa, μs) for the blood seeded with different tracer particles materials at different laser wavelengths. The best results demonstrates that the scattering coefficients for human blood were 89.81 1/mm seeded with silver particles using blue laser operating at wavelength of 457 nm were investigated under laboratory conditions.

10063-2, Session 1

Transcranial optical vascular imaging (TOVI) during cardiac arrest *(Invited Paper)*

Vyacheslav Kalchenko, Yuri Kuznetsov, Weizmann Institute of Science (Israel); Igor Meglinski, Univ. of Oulu (Finland); Alon Harmelin, Weizmann Institute of Science (Israel)

Based on the recent studies the prognosis of patients after cardiac arrest (CA) remains poor. Thus it is extremely important to understand fine mechanisms related to the influence of CA on the brain and Cerebral Blood Flow (CBF) during and after cardiac arrest. Recently our group introduced Transcranial Optical Vascular Imaging (TOVI) approach that combines laser speckle and dynamic fluorescent imaging. TOVI proved to be useful during various preclinical brain research applications. For example it allows imaging of brain blood vessels of a mouse in vivo through the intact cranium. Herein for the first time we present the use of TOVI during cardiac arrest. TOVI possibly could be a useful tool for preclinical studies of CBF during and after CA.

10063-3, Session 1

Laser Doppler flowmetry in blood and lymph monitoring, technical aspects and analysis

Victor V. Dremin, Orel State Univ. named after I.S. Turgenev (Russian Federation); Evgeny A. Zherebtsov, Orel State Univ. named after I.S. Turgenev (Russian Federation) and Univ. of Oulu (Finland); Irina N. Makovik, Igor O. Kozlov, Orel State Univ. named after I.S. Turgenev (Russian Federation); Viktor V. Sidorov, SPE LAZMA Ltd. (Russian Federation); Alexander I. Krupatkin, Priorov Central Research Institute of Traumatology and Orthopaedics (Russian Federation); Andrey V. Dunaev, Orel State Univ. named after I.S. Turgenev (Russian Federation); Karina S. Litvinova, Ilya E. Rafailov, Sergei G. Sokolovski, Edik U. Rafailov, Aston Univ. (United Kingdom)

At present, laser Doppler flowmetry (LDF) is a method widely used in diagnosis of microcirculatory diseases. The approach links integral characteristics of the photocurrent's power spectrum with the average concentration and velocity of red blood cells (RBCs) in a sampling volume of tissue. In this research, it is proposed to compute the integrals (indexes of microcirculation) in the sub-ranges of the power spectrum.

Applying standard physiological functional tests to a limb results in changing RBC velocity distribution inside the skin. Several series of experiments on exclusively healthy volunteers were performed in order to record such alternations during breath tests. A single mode 1064 nm laser was selected as the source of sounding radiation. Optical fibres were used to deliver radiation to the skin and to collect backscattered light. The signal was processed LabVIEW (National Instruments, USA). Parts of power spectra in frequency ranges of 0 – 200 Hz, 200 – 400 Hz, 400 – 800 Hz, 800 – 1600 Hz, 1600 – 3200 Hz, and 3200 – 6400 Hz were simultaneously obtained from fingers and subsequently analysed. Appropriate hardware and software were developed.

Processing of the obtained experimental data has shown that the Doppler power spectrum undergoes alterations during the breath test. These experiments revealed a dramatically decreasing signal in the higher frequency ranges corresponding to the velocity of the RBCs in arterioles. Furthermore, lymphatic flow alterations, mirroring those of blood flow, were observed in the lower frequency ranges. The above mentioned differences and alterations in lymph and blood flows seemingly demonstrate correlation.

10063-4, Session 1

Modeling and interpreting speckle pattern formation in swept-source optical coherence tomography

Valentin Demidov, Univ. of Toronto (Canada); I. Alex Vitkin, Univ. of Toronto (Canada) and Princess Margaret Cancer Ctr., Univ. Health Network (Canada); Alexander Doronin, Yale Univ. (United States); Igor Meglinski, Univ. of Oulu (Finland)

We report on the development of a unified Monte-Carlo based computational model for exploring speckle pattern formation in swept-source optical coherence tomography (OCT). OCT is a well-established optical imaging modality capable of acquiring cross-sectional images of turbid media, including biological tissues, utilizing back scattered low coherence light. The obtained OCT images include characteristic features known as speckles. Currently, there is a growing interest to the OCT speckle patterns due to their potential application for quantitative analysis of medium's optical properties. Here we consider the mechanisms of OCT speckle patterns formation for swept-source OCT approaches and introduce further developments of a Monte-Carlo based model for simulation of OCT signals and images. The model takes into account polarization and coherent properties of light, mutual interference of back-scattering waves, and their interference with the reference waves. We present a corresponding detailed description of the algorithm for modeling these light-medium interactions. The developed model is employed for generation of swept-source OCT images, analysis of OCT speckle formation and interpretation of the experimental results. The obtained simulation results are compared with selected analytical solutions and experimental studies utilizing various sizes / concentrations of scattering microspheres.

10063-5, Session 1

Unobtrusive monitoring of heart rate using a cost-effective speckle-based SI-POF remote sensor

Carmen Vázquez García, Plinio Jesús Pinzón Castillo, David S. Montero, Alberto Tapetado Moraleda, Univ. Carlos III de Madrid (Spain)

Irregular heartbeat or arrhythmias are very common. Occasionally, the heart beats too fast or too slow which it is not necessarily a symptom of any disease. However, if it happens frequently or for a long period it may be due to any cardiovascular disease (CVD). According to the World Health Organization, CVDs killed 17.5 million people in 2012, representing 31% of all global deaths, and three quarters of these deaths occurred in low-and middle-income countries.

Heart rate monitoring can be based on mechanical activity of cardiac muscle. To date, many fiber Bragg grating based sensors as well as Michelson interferometers have been tested for this purpose, but their associated costs are high. However, for a wider usage of in-home and in-office healthcare systems, less expensive solutions are needed, especially when considering that people spend on average almost 6 h per day sitting at their desk and 7 h sleeping at night. In this scenario, speckle interferometry is one of the possible alternative approaches for a cost-

effective heart rate monitoring, as shown recently, in schemes with multiple contacts and in unobtrusive schemes.

Optical sensing features include small size, light weight, geometrical flexibility, chemical inertness, electric and thermal insulation, and immunity to electromagnetic interference. Additionally, Plastic Optical Fibers (POFs) may overcome biocompatibility concerns as well as provide multiple applications in sensing systems at a very low or competitive cost compared to the well-established conventional technologies. In this work a novel speckle-based sensing technique for cost-effective heart-rate monitoring is proposed. The proposed technique detects periodical changes in the spatial distribution of energy on the speckle pattern in the output of a Step-Index-POF lead. The scheme operates in reflective configuration thus performing a centralized interrogation unit scheme. The prototype has been tested on 5 patients lying on a bed in different positions without direct contact with the sensor cable.

10063-6, Session 1

Optical vortices as potential indicators of biophysical dynamics

Anindya Majumdar, Sean J. Kirkpatrick, Michigan Technological Univ. (United States)

Speckles are granular patterns produced as a result of random interference of light waves. Optical vortices (OVs) are phase singularities in such speckle fields, characterized by zero intensity and an undefined phase. Decorrelation of the speckle fields causes these OVs to move around. In this work, a variety of parameters of these OVs have been studied. The speckle fields were simulated to undergo three distinct decorrelation behaviors- Gaussian, Lorentzian and Constant decorrelations. Different decorrelation behaviors represent different dynamics. For example, Lorentzian and Gaussian decorrelations represent Brownian and steady motions, respectively. Typical dynamical systems in biophysics are generally argued to be a combination of these. For each of the decorrelation behaviors under study, the vortex trails were tracked by varying the rate of decorrelation. Parameters such as the decorrelation length, average trail length and the deviation of the vortices as they traversed in the speckle field, were studied. Empirical studies were also performed to define the distinction between two separate trails. The initial studies under stationary speckle fields were followed up by similar studies on shifting fields. A new idea to employ Poincare plots in speckle analysis has also been introduced. Our studies indicate that tracking OVs can be a potential method to study cell and tissue dynamics.

10063-7, Session 2

Characteristics of blood components: markers of diseases as assessed by optical techniques *(Invited Paper)*

Alexander V. Priezzhev, M.V. Lomonosov Moscow SU (Russian Federation)

This paper focuses at the characteristics of blood that can be measured in-vitro and/or in-vivo by laser-optic techniques, and which alterations can be considered as an indication of disease. The techniques to be discussed are: laser diffractometry of erythrocytes, laser scattering aggregometry of erythrocytes, digital optical capillaroscopy and measurement of the microcirculation parameters, erythrocytes trapping and manipulation with laser tweezers, fluorescence spectroscopy of blood plasma, etc. Blood for the experiments in-vitro was drawn from clinically healthy human volunteers (control) and from patients suffering from diabetes mellitus, hypertension and other diseases. In-vivo measurements of the microcirculation parameters were conducted with control human subjects and patients suffering hypertension. For the in-vitro experiments with rat blood, the samples were drawn from healthy (control) and sham operated animals, those with experimentally induced diabetes mellitus and/or hypertension. Samples of human and rat blood plasma separated from blood cells were used to study the aggregation of plasma proteins that leads to the loss of their functional properties and is one of the causes of socially important diseases. Fluorescence spectroscopy allows also for identifying other alterations at the molecular level in blood plasma caused by proteins conformational changes that may induce pathological changes at the cellular level and so on up to the level of the whole organism.

The obtained results allow us to conclude that the overviewed laser-optic techniques comprise a powerful tool for efficiently identifying and assessing a set of clinically informative bio-optical markers of diseases.

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10063-8, Session 2

Multi-exposure speckle imaging of cerebral blood flow: a pilot clinical study

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Monitoring cerebral blood flow (CBF) during neurosurgery is essential for detecting ischemia in a timely manner for a wide range of procedures. Multiple clinical studies have demonstrated that laser speckle contrast imaging (LSCI) has high potential to be a valuable, label-free CBF monitoring technique during neurosurgery. LSCI is an optical imaging method that provides blood flow maps with high spatiotemporal resolution requiring only a coherent light source, a lens system, and a camera. However, the quantitative accuracy and sensitivity of LSCI is limited and highly dependent on the exposure time. An extension to LSCI called multi-exposure speckle imaging (MESI) overcomes these limitations, and was evaluated intraoperatively in patients undergoing brain tumor resection. This clinical study (n = 7) recorded multiple exposure times from the same cortical tissue area, and demonstrates that shorter exposure times (≤1 ms) provide the highest dynamic range and sensitivity for sampling flow rates in human neurovasculature. This study also combined exposure times using the MESI model, demonstrating high correlation with proper image calibration and acquisition. The physiological accuracy of speckle-estimated flow was validated using conservation of flow analysis on vascular bifurcations. Flow estimates were highly conserved in MESI and 1 ms exposure LSCI, with percent errors at 6.4% ± 5.3% and 7.2% ± 7.2%, respectively, while 5 ms exposure LSCI had higher errors at 21% ± 10% (n = 14 bifurcations). Results from this study demonstrate the importance of exposure time selection for LSCI, and that intraoperative MESI can be performed with high quantitative accuracy.

10063-9, Session 2

Optical diagnosis of cervical cancer by intrinsic mode functions

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In this paper, we make use of the empirical mode decomposition (EMD) to discriminate the cervical cancer tissues from normal ones based on elastic scattering spectroscopy. The phase space has been reconstructed through decomposing the optical signal into a finite set of bandlimited signals known as intrinsic mode functions (IMFs). The analytic signal representation of IMFs

is obtained by the Hilbert transformation of IMFs. The area measured from the trace of the analytic IMFs, which have circular form in the complex plane, and the extent of deviation from circular to elliptic structure for cancerous ones from Normal ones is striking feature for pre-cancer detection. It has been shown that the area measure of the analytic IMFs provides a good discrimination performance. Simulation results validate the efficacy of the EMD based methodology for classification.

10063-10, Session 2

Algorithmic processing of dual intrinsic signals in affixed transmission speckle analysis (ATSA)

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Affixed Transmission Speckle Analysis (ATSA) is a method recently developed to measure blood flow that is based on laser speckle imaging miniaturized into a clip-on form factor the size of a pulse-oximeter. Measuring at a rate of 250 Hz, ATSA is capable or obtaining the cardiac waveform in blood flow data, referred to as the Speckle-Plethysmogram (SPG). ATSA is also capable of simultaneously measuring the Photoplethysmogram (PPG), a more conventional signal related to light intensity. In this work we present several novel algorithms for extracting physiologically relevant information from the combined SPG-PPG waveform data. First we show that there is a slight time-delay between the SPG and PPG that can be extracted computationally. Second, we present a set of frequency domain algorithms that measure harmonic content on pulse-by-pulse basis for both the SPG and PPG. Finally, we apply these algorithms to data obtained from a set of subjects including healthy controls and individuals with heightened cardiovascular risk. We hypothesize that the time-delay and frequency content are correlated with cardiovascular health; specifically with vascular stiffening.

10063-11, Session 2

Eigendecomposition-based algorithm for optical microangiography in dermatology

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Optical coherence tomography (OCT)-based microangiography (OMAG) is increasingly becoming a powerful imaging tool to visualize the microcirculation in human skin. Nowadays a number of algorithms have been reported to achieve OCT angiography which can be categorized into amplitude-based, phase-based and complex-based approaches. Imaging of human skin in vivo is challenging due to the intrinsic bulk motion of human body and the highly optical scattering of skin tissue. Here we demonstrate a complex-based algorithm of eigendecomposition-based optical microangiography (ED-OMAG) for the imaging of human skin vasculature. The data of OCT signal was acquired by a high-speed swept-source OCT system at 1310 nm central wavelength with 100 kHz A-line rate. By comparing with the speckle variance approach which is an amplitude-based algorithm, ED-based angiography provides superior imaging performance with higher contrast and sensitivity for cutaneous blood flow visualization due to the fully utilization of both amplitude and phase information of the OCT signal. Moreover, ED-OMAG is less sensitive to bulk movement of human body thus the motion artifact in the vascular image is alleviated. Without the necessary of motion correction and phase compensation, ED-OMAG provides us high computation efficiency for vascular imaging. In this study, the proposed ED-OMAG has been successfully applied to visualize the richness of blood vessels from superficial layer to deep dermis layer in human skin, which is expected to benefit the clinical dermatology application.

10063-1, Session 3

Nanobiophotonics breaks lymphatic theranostic challenges *(Keynote Presentation)*

Ekaterina I. Galanzha, Univ. of Arkansas for Medical Sciences (United States)

We introduce a multimodal (photoacoustic–photothermal-fluorescence-Raman) lymph flow cytometry to provide a major advance in the challenging area of in vivo single cell analysis of the lymphatic system. Recent development of this platform includes new-synthetized nanoparticles as high contrast photoacoustic and photothermal contrast nanoagents, multicolor laser arrays, high speed signal acquisition algorithms, and multiplex targeting by conjugated nanoparticles and fluorescent proteins for molecular labeling and genetic encoding, respectively. Based on our preclinical results, we present the capability of this technical platform to integrate, for the first time to our knowledge, highly sensitive diagnosis of cells in lymph flow and lymph nodes and targeted their therapy (killing), if cells lead to diseases. Since lymphatic system is a key role in many severe diseases (e.g., metastatic cancer, lymphedema and malformation), we discuss future translation from bench to bedside that is expected by using clinically-relevant photoacoustic lymph flow cytometry in label-free (i.e., non-toxic) mode or using low-toxic nanoparticles.

10063-12, Session 4

A new full-field interferometry approach for counting and differentiating aquatic biotic nanoparticles *(Invited Paper)*

A. Claude Boccara, Yasmina Fedala, Justine Voronkoff, Institut Langevin (France); Nina Paffoni, Martine Boccara, Institut de Biologie de l'École Normale Supérieure (France)

Due to the huge abundance and the major role that viruses and membrane vesicles play in the seas or rivers ecosystems it is necessary to develop simple, sensitive, compact and reliable methods for their detection and characterization. Our approach is based on the measurement of the weak light level scattered by the biotic nanoparticles. We describe a new full-field, incoherently illuminated, shot-noise limited, common-path interferometric detection method coupled with the analysis of Brownian motion to detect, quantify, and differentiate biotic nanoparticles. The last developments take advantage of a new fast (700 Hz) camera with 2 Me- full well capacity that improves the signal to noise ratio and increases the precision of the Brownian motion characterization. We validated the method with calibrated nanoparticles and homogeneous DNA or RNA.viruses. The smallest virus size that we characterized with a suitable signal-to-noise ratio was around 30 nm in diameter with a target towards the numerous 20 nm diameter viruses. We show for the first time anisotropic trajectories for myoviruses meaning that there is a memory of the initial direction of their Brownian motions. Significant improvements have been made in the handling of the sample as well as in the statistical analysis for differentiating the various families of vesicles and virus. We further applied the method for vesicles detection and for analysis of coastal and oligotrophic samples from Tara Oceans circumnavigation as well of various rivers.

10063-13, Session 4

Quantification of mammary organoid toxicant response by OCT fluctuation spectroscopy

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OCT fluctuation spectroscopy provides a quantitative and non-invasive tool to monitor 3D mammary epithelial cell (MEC) models which recapitulate features of breast cancer in vivo. Cellular dynamic fluctuation measurements allow for assessing drug responses due to different mechanisms of inhibition in 3D models in vitro. Here we report on pre-malignant MEC responses to two toxicants: Taxol, which stabilizes microtubules against depolymerization, and has emerged as an important chemotherapeutic agent in the treatment of breast cancer, and blebbistatin, a small molecule inhibitor with high affinity and selectivity toward Myosin II. Here we propose a quantitative method for measuring toxicant responses of MECs in 3D cultures from OCT speckle fluctuation spectroscopy. OCT fluctuation spectra were quantified within the 9-440 mHz band, from which the inverse power-law exponent (?) and the fractional modulation amplitude (M) were extracted. Pre-malignant MECs treated with Taxol (0, 10 ?M and 20 ?M) exhibit an increase ($p<0.001$) in ? along time (t=0, 1 hour, 24 hours, 48 hours and 6 days), indicating selective inhibition of high frequency fluctuations, with a concomitant decrease ($p<0.001$) in M over time. However, only M of pre-malignant MECs treated with Blebbistatin (0, 25 uM and 50 uM) exhibited a decrease ($p<0.001$) along time, but no significant change ($p>0.05$) in ?, since myosin II isn't highly expressed in non-migratory pre-malignant MECs. We demonstrated the feasibility of OCT fluctuation spectroscopy to quantify MEC toxicant response. Microtubule stabilization was found to be a major contributor to pre-malignant MEC fluctuation signals, while myosin II inhibition was not.

10063-14, Session 4

Using OCT-based microangiography for in vivo longitudinal study of arteriogenesis

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The adaptive growth of collateral vessels, termed "arteriogenesis", is crucial for maintaining regional blood supply during arterial obstruction and offsetting the adverse effect of tissue ischemia. Stimulation of arteriogenesis has been applied for the treatment of occlusive vascular diseases, and in vivo imaging of the progressive development of collateral vessel will facilitate a better understanding of the mechanism. We present using high-resolution OCT-based microangiography (OMAG) to image arteriogenesis process longitudinally in mouse cerebral cortex after middle cerebral artery occlusion (MCAO). We imaged the collateral arterioles at the arteriolo-arteriolar anastomosis (AAA) within 7-day period after MCAO to reveal key elements of collateral vessel remodeling, including alteration in vessel morphology, velocity and directionality of blood flow. The magnitudes of changes in these parameters matched the time course of the active building of collateral vessels stated in previous studies using histology. Hence, OMAG is a promising imaging tool for non-invasive longitudinal study of functional collateral vessel growth in small animal models and can be potentially applied in the experimental study of arteriogenesis stimulation.

10063-31, Session PSun

Measurement of oxygen-saturation with dual beam photothermal optical coherence tomography

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Optical coherence tomography (OCT) is a high resolution imaging technology that is used for in vivo cross sectional and 3 D imaging of microstructure in biological tissues. Conventional OCT imaging is based on contrast from spatial variations in tissue scattering that exploits the changes in refractive index within the imaged sample. Apart from obtaining structural information within the sample, functional extensions of OCT have also been developed. Phothothermal OCT (PT-OCT) is one of the functional extensions of OCT that is capable of imaging endogenous and exogenous contrast agents. PT-OCT uses a modulated laser beam to heat the tissue volume being scanned. The modulated heating induces periodic phase shift that is detected by OCT. In this work, feasibility of measuring oxygen saturation using PT-OCT is demonstrated. This technique uses a spectral domain OCT system together with two photothermal beams that are selectively absorbed by oxyhaemoglobin (O2Hb) and unbound haemoglobin (Hb). As proof of concept, experiments were first carried out using absorptive dyes in capillary phantom models. In addition, oxygen saturation measurements were carried out on capillary phantoms filled with blood. A technique was developed which enables simultaneous measurement of blood flow and the oxygen gradient, thereby providing the oxygen consumption rate.

10063-32, Session PSun

Influence of cost-functions and optimization methods on the efficiency of solving the inverse problem in diffuse reflectance

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Diffuse reflectance spectroscopy (DRS) is a promising optical biopsy tool for cancer detection. In spatially resolved DRS, accurate estimation of the optical parameters of biological tissues is a major challenge due to the complexity of the physical models. Solving this inverse problem heavily depends on three factors: the forward model, the cost function, and the optimization algorithm.

This paper presents a numerical study aiming to facilitate the choice of the cost function and the related optimization algorithm. For the forward model, one- and two-layer diffusion approximation models are implemented as well as fast Monte Carlo simulations. Noise is added to these input data in order to evaluate the robustness of estimation. As for the inverse problem, the considered cost functions possibly include normalized data terms and regularization terms promoting spectral smoothness. Two local optimization methods, Levenberg-Marquardt and Trust-Region-Reflective, are considered. Because they may be sensitive to the initial setting, a global optimization approach is proposed to improve the estimation accuracy.

This algorithm is based on repeated calls to the above-mentioned local optimization methods, with randomly sampled initial parameters.

Numerical methods are evaluated in terms of relative errors between the ground truth and the estimated values of unknown parameters. The shape of each cost function is analyzed in order to identify the level of difficulty of parameter estimation. This should help the practitioner to choose an appropriate combination between the number of variables to be estimated, the nature of the forward model, the cost function and the optimization method.

10063-33, Session PSun

New photoswitchable hybrid nanocomposite of gold and fluorescent proteins for advanced biomedical applications

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The ability to remotely change the optical properties of molecular probes can provide valuable insight to the dynamics of intra- and inter-cellular movement and distribution. Genetically engineered cells producing photoswitchable fluorescence proteins have already proven to be a valuable biological tool, providing many discoveries in neuroscience and allowing the tracking of single circulating tumor cells in the bloodstream. However, in many cases weak fluorescence prevents study cells in deep tissue and cannot be used for multimodal detection. We introduce photoswitchable probes consisting of photoswitchable fluorescent proteins Dendra2 embedded in porous silica layer around a spherical gold core. In addition to fluorescence protein detection, the strong absorption of the gold core can also be used for photoacoustic and photothermal detection. We demonstrated photoswitching of these probes with simultaneous fluorescence and photoacoustic detection, verified with conventional and electron microscopy. We also confirmed that Dendra2 has unique spectrum before and after switching using fluorescence spectroscopy. The advantage of these probes lies in using pre-synthesized photoswitchable protein in tandem with nanoparticles in effort to label cells without the need of genetic manipulation. Being able to modulate the absorbance of gold core nanoparticles via light would allow these probes to be used as photoacoustic contrast agents for the visualization and tracking of single circulating bacteria or cancer cells in the body.

10063-34, Session PSun

Recurrence quantification as potential bio-markers for diagnosis of pre-cancer

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The analysis of the non-linear trajectories of cancerous and normal tissues arising from treating the intensity signals as a dynamical time series is of considerable interest. It is difficult to decipher the dynamics in these signals by visual inspection and other linear techniques. Hence, non-linear methods like recurrence quantification has been deployed here to analyze the optical spectroscopic data. In this work, the spectroscopy signals have been analyzed in recurrence plots (RP) and extract recurrence quantification analysis (RQA) parameters from the RP in order to classify the tissues into normal and different precancerous grades. Recurrence plot (RP) is a graph that shows a recurrence state of the dynamical system. Hence, three RQA parameters have been quantified in order to extract the important features in the spectroscopy data. These features have been fed to different classifiers for classification. Simulation results validate the efficacy of the recurrence quantification as potential bio-markers for diagnosis of pre-cancer.

10063-35, Session PSun

Continuous blood pressure recordings simultaneously with functional brain imaging: studies of the glymphatic system

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The lymph system is responsible for cleaning the tissues of metabolic waste products, soluble proteins and other harmful fluids etc. Lymph flow in the body is driven by body movements and muscle contractions.

Moreover, it is indirectly dependent on the cardiovascular system, where the heart beat and blood pressure maintain force of pressure in lymphatic channels. Over the last few years, studies revealed that the brain contains the so-called glymphatic system, which is the counterpart of the systemic lymphatic system in the brain. Similarly, the flow in the glymphatic system is assumed to be mostly driven by physiological pulsations such as cardiovascular pulses. Thus, continuous measurement of blood pressure and heart function simultaneously with functional brain imaging is of great interest, particularly in studies of the glymphatic system.

We present our MRI compatible optics based sensing system for continuous blood pressure measurement and show our current results on the effects of blood pressure variations on cerebral brain dynamics, with a focus on the glymphatic system. Blood pressure was measured simultaneously with near-infrared spectroscopy (NIRS) combined with an ultrafast fMRI sequence magnetic resonance encephalography (MREG, 3D brain 10 Hz sampling rate).

10063-36, Session PSun

Impact of blood volume changes within the human skin on the diffuse reflectance measurements in visible and NIR spectral ranges

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We consider changes in the volume of blood and oxygen saturation caused by a pulse wave and their influence on the diffuse reflectance spectra measured in the visible/NIR spectral range. A CUDA-based Monte-Carlo online platform was used for routine simulation of detector depth sensitivity (sampling volume) and skin reflectance spectra, as well as their variations associated with physiological changes in the human skin. The computational model of skin utilizes 7 to 18 layers. The variations in the spatial distribution of blood, melanin, oxygen saturation of blood, hematocrit, water content within the skin, as well as the numerical aperture and angle of the detector positioning on the skin surface are taken into account. The results are presented in the form of animated graphs of sampling volume changes for scaling of the parameters of the main human skin layers related to the results of experimental measurements. The results of the study are of a particular interest for pulse oximetry, photoplethysmography, laser Doppler flowmetry, and diffuse reflectance spectroscopy.

10063-37, Session PSun

Optical phantoms to mimic fluid dynamics in the brain

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Emerging neuroimaging techniques and rapid progress in theoretical and experimental methods during the recent years might lead to great achievements in the overall knowledge of the human brain. In these studies, optics based techniques play a growing role since they can support more commonly used brain imaging techniques, particularly electroencephalogram (EEG) and magnetic resonance imaging (MRI), and provide additional information, for instance, for fluid dynamics in the brain. However, optical brain imaging techniques, such as near-infrared spectroscopy (NIRS), still need further development and improvements in their sensitivity and spatial accuracy. To accomplish this and to experimentally validate the developed techniques, use of optical phantoms of the brain tissues is essential.

We present a developed multi-layered optical tissue phantoms to support the development of optics based measurements of the brain. We focus on creating liquid flows inside the phantoms to mimic fluid dynamics in the brain. The solid phantoms are made from a silicone elastomer polydimethylsiloxane (PDMS) and glycerol mixture and dried haemoglobin. In those phantoms we do not use scattering nanoparticles, where scattering is achieved by the material itself: spherical cavities trapped in a silicone matrix as well as with addition of haemoglobin.

10063-38, Session PSun

Intermittent behavior in the brain neuronal network in the perception of ambiguous images

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In the present report we consider the characteristics of intermittent behavior in the process of bistable visual perception. As intermittency the alternation between two different projections of an ambiguous image is considered. As an ambiguous image Necker cube illusion demonstrating two projections, the left-oriented and the right-oriented cube, namely, has been chosen. The contrast of the three middle lines centered in the left middle corner, 0< I<1, was used as a control parameter. The contrast of the three middle lines centered in the right middle corner was set to (1-I), and the contrast of the six visible outer cube edges was fixed to 1. Time of observation of one of the cube projection has been detected and the distribution of time interval lengths corresponding to the period of perception of the projection has been obtained for different values of the control parameter I and several subjects. An average lengths of time intervals during which one observes each projection of Necker cube has been also calculated. Mathematical approach based on the stochastic differential equation with the bistable potential for the interpretation of obtained results has been developed. This model takes into account the influence of cognitive noise on the process of bistable visual perception. Our theoretical and experimental results are in good agreement with each other.

10063-39, Session PSun

Polarization enhanced laser speckle contrast imaging for vascular imaging

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Laser speckle contrast imaging(LSCI) is a simple inexpensive optical imaging modality that can in-vivo image vascular vessels with high contrast and large field of view. Speckle is formed by light interference. The polarization of light plays an important role in the forming of the speckle imaging. This study is to determine how the polarization state of light affects the imaging contrast in laser speckle contrast imaging of blood vessels. Four types of polarization laser beam including horizontal polarization, vertical polarization, left-hand circular polarization and right-hand circular polarization are used to illuminate a mimic blood vessel embedded near subsurface in a tissue mimic turbid phantom for LSCI imaging. The imaging contrast by each incident polarization state is studied. The effects of the flow rate and camera exposure time to the imaging contrast are also studied. In addition, background subtraction effect to the image contrast under each incident polarization is also studied. Background subtraction is obtained through the subtraction between two images formed by two orthogonal polarization states based on the incident polarization. Overall, the purpose of this study is to understand the polarization effects in LSCI imaging and help to improve the imaging contrast in LSCI imaging modality.

10063-40, Session PSun

Intermittent phase synchronization in human epileptic brain

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One of the most interesting types of the synchronous behavior observed in physiological systems in the phase synchronization regime. It is the generalization of classical synchronization of periodical oscillations on the case of non-autonomous or coupled chaotic systems and means the presence of the phase locking of interacting systems in the absence of any correlations of their amplitudes. Near the boundary of the phase synchronization the intermittent behavior is observed. In such case the phase locking condition is satisfied only in certain time intervals called by the laminar phases, which are persistently interrupted by the phase slips called by the turbulent phases. Such regime is called by the intermittent phase synchronization. Intermittent phase synchronization is a generic type of the synchronous behavior observed both in physical, biological and physiological systems.

In present report we find the intermittent phase synchronization in human epileptic brain. We show that the laminar phases can be observed both during the epileptic seizures and in the fields of the background activity of the human brain. We estimate the degree of intermittent phase synchronization in both considered cases and found that the epileptic seizures are characterized by the higher degree of synchronization in comparison with the fields of background activity. For estimation of synchronization degree we propose the modification of the method for estimation of zero conditional Lyapunov exponent from time series described in [PRE 92 (2015) 012913].

10063-41, Session PSun

Recognition and classification of oscillatory patterns of electric brain activity using artificial neural network approach

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Experimental methods have recently been developed for registering the neuronal activity underlying processes of information encoding–decoding by means of changes in the local (electrical) field potentials in the cerebral cortex of brain. Traditional and noninvasive methods for registering electrical brain activity, such as electroencephalography (EEG) with electrodes arranged on the head skin, offer several advantages, and this method is still commonly used in neurophysiology and medicine. To identify the important features of mentality and decision-making of human we have studied the reactions on the so-called optical illusions, such as the Necker cube, which can be perceived ambiguously (left-hand and right-hand cubes). Standard 10-20 EEG recording system has been used to record the electrical activity.

This report presents the results of the artificial neural network (ANN) development to analyze the possibility of determining the brain activity patterns corresponding to a particular perception of the left-hand/right-hand Necker cube. We have analyzed the fragments of multi-channel EEG with duration of 750 samples (corresponding to the 3 s with 250 Hz sample rate), taken after the presentation of the ambiguous image. We have shown that developed and trained ANN for an all recorded EEG allows us to identify left-hand/right-hand Necker cube perception with the probability close to 80%. We have also estimated the quality of classification for different EEG channels combinations and revealed ones for which the performance of ANN was not less than 75%. So, the provided analysis reveals the most significant EEG channels demonstrates the possibility of reducing the channels number to be used without degradation of the classification quality of ambiguous image perception using ANN.

10063-42, Session PSun

Filtration of human EEG recordings from physiological artifacts with empirical mode methods

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Now EEG methods are one of the leading ways to register human brain activity in the cognitive processes. However, EEG recording is registered the set of interference and noise with the desired signal. In this paper, we present a way to dealing with noise and physiological artifacts in human EEG recordings based on empirical mode method. The method of Empirical Mode Decomposition is based on the decomposition of signal into several components (Empirical Mode) by calculating the signal envelope. For human EEG data interference and the useful signal are well divided into different components. In this paper, we show that by using only the first mode we save 95% of the information of the desired signal in the frequency range with the alpha rhythm beginning and beyond. We are considering the possibility of removing the oculomotor and cardiac artifacts in the EEG method. We compare the results of the method of empirical mode and the method by the Gram-Schmidt transforming to remove oculomotor artifacts. Recovering EEG data with using empirical mode and suppression of physiological artifacts does not require the registration of additional signals - oculogram, electrocardiogram, etc. This technique can also be applied when conducting routine psychophysiological works when the imposition of the additional electrodes is not justified.

10063-43, Session PSun

The study of cognitive processes in the brain EEG during the perception of bistable images using wavelet skeleton

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This report presents the results of experimental studies and mathematical processing of the perception process of ambiguous images. Necker cube is chosen as a model of this image type. Experimental design has included irregular presentation of Necker cube various modifications to a volunteer. Volunteers have marked their first perception of the object by pressing one of the buttons of a special pult. Volunteer group has consisted of about 30 healthy people. Standard non-invasive 10-20 EEG recording system has been used to record the brain electrical activity. We offer a method based on the wavelet skeleton calculating for processing EEG records. The calculation of the main three or four skeletons can estimate the dynamics of the basic processes, recorded in different leads. We have discovered the space techniques of visualization skeletons data in time for all electrodes system. We have presented a developed method for objective and automatic estimate the presence and features of a particular oscillatory activity in the multichannel EEG data.

The developed methods have been used for EEG data studying to a group of subjects. Different patterns in the oscillatory activity of the frontal and occipital leads have detected in automatic regime. We present that on the basis of the discovered regularities we can determine the time of presentation cubes and moments of decision-making by volunteers with a high degree of accuracy (from 60 to 90% for different subjects).

10063-44, Session PSun

Temperature sensing of adipose tissue heating with the luminescent upconversion nanoparticles as nanothermometer: in vitro study

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The luminescence spectra of upconversion nanoparticles (UCNPs) were measured and analyzed in a wide temperature range: from room to human body and further to a hyperthermic temperature resulting in tissue morphology change.

The two types of synthesized UCNP [NaYF: Er, Yb] structures, namely, powdered and as a polymer film with embedded particles, were used.

An aqueous suspension of UCNPs was used. Typical samples thickness of adipose tissue was 1.4 mm. The sample temperature measured by a thermocouple varied between 25 °C and 60 °C. The temperature dependence of the UCNPs luminescence intensity from UCNPs deposited onto the sample surface (1 layer) and from UCNPs deposited between two layers of human adipose tissue was recorded.

The results show that the luminescence signal of UCNPs placed within the tissues is reasonably good sensitive to temperature change and accompanied by phase transitions of lipid structures of adipose tissue. The most likely that the multiple phase transitions are associated with the different components of fat cells, such as phospholipids of cell membrane and lipids of fat droplets. In the course of fat cell heating, lipids of fat droplet first transit from a crystalline form to a liquid crystal form and then to a liquid form, which is characterized by much less scattering. The obtained results confirm a high sensitivity of the luminescent UCNPs to the temperature variations within tissues and show a strong potential for the controllable tissue thermolysis.

10063-45, Session PSun

Low-level laser therapy on chondrocyte viability

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INTRODUCTION: Osteoarthritis (OA), the most prevalent form of arthritis, is a chronic and painful disease of synovial joints, most commonly the knees, hips, and hands. It is characterized by gradual degeneration of the joint, progressive destruction of articular cartilage and new bone formation at the joint surface and surrounding areas (Cytokine 70(2):185-93). Due to the very limited cartilage regenerative capacity and consequently the limited efficacy of the standard treatments, the investigation of strategic innovative approaches to prevent the development of the clinical condition of OA is of great interest (World J Orthop 18; 5(3): 351-361). One promising treatment is the use of low-level laser therapy (LLLT) mainly due to its anti-inflammatory and regenerative effects on biological tissues (Lasers Surg Med 2004;35(3):229–35). Therefore, this study aimed to investigate the effects of LLLT on chondrocytes viability. METHODS: This study was approved by the Animal Care Committee guidelines at Federal University of São Paulo (CEUA N 2478130315). Chondrocytes were obtained from collagenase-digested femoral growth plate cartilage of 7-week-old rats. Chondrocytes were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco BRL, Life Technologies) supplemented with 10% fetal bovine serum (FBS; Gibco). All tissue culture procedures were performed under strict aseptic conditions in a biological safety cabinet. Chondrocytes were grown in sterile, vented, 175 cm2 tissue culture flasks (Greiner Bio-one, Utrecht, the Netherlands) in a humidified incubator at 37°C in 5% carbon dioxide (CO2), 95% air. The cells were expanded for four passages using standard tissue culture techniques. Subsequently, Chondrocytes were seeded in direct contact with the particles at a density of 5.104 cells/well. Cells were cultured for 3 days. Cell irradiation was performed using a Photon Laser III - DMC Equipament®, with a wavelength of 808 nm, 50 mW, 30 J/cm2, 16 seconds, 0,8 J. The cells were irradiated 24 h after seeding, for three consecutive days, once every 24 h, on 48-well culture plates. The action of the laser's biomodulation was evaluated in three experimental groups: G1—control group, which received no irradiation and G2—irradiated at 30 J/cm2. The irradiation was performed shielded from the light in a darkened room, with the laser pointer tip in direct contact with the plate. The experiment was conducted in triplicate. Cell metabolic activity was evaluated using Alamar Blue® (Invitrogen, Life Technologies), according to the manufacturer's instructions. Subsequently, 200 ?l of each sample was transferred to a 96 well plate (in duplicates). Finally, the plate was read in a spectrophotometer (Bio-Tek Instruments, Winooski, USA) at 570 nm. RESULTS: The alamarBlue® assay is based on the ability of metabolically active cells to convert the reagent into a fluorescent and colorimetric indicator. Thus, the results revealed that the cell metabolic activity was significantly higher for LLLT compared to control group (p< 0,0026) which are evidenced by the appearance a large number of live cells after 3 days. CONCLUSIONS: Thus, based on the results of the present study, it has been shown that LLLT within the parameters presented is capable of stimulating the Chondrocytes viability.

10063-46, Session PSun

Pattern formation in adaptive multiplex network in application to analysis of the complex structure of neuronal network of the brain

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Synchronization is a fundamental nonlinear phenomenon occurring in systems of interacting units, and is ubiquitous in nature, living systems, society and technology. Recent studies of complex networks have enlightened the key role played by the interaction topology on the emergence of synchronized states. In the report we study the mechanisms and features of pattern formation in the adaptive multiplex network of Kuramoto phase oscillators. We have considered multiplex network with two layers with 300 phase oscillators on each. The interaction between the nodes (oscillators) on each layer in considered adaptive network [Scientific Reports, vol. 1:99, pages 1-8, 2011] is defined by the principles of competition. i.e., graph of links between nodes of network co-evolves with the dynamical process of synchronization of nodes leading to increasing of coupling between asynchronous oscillators.

Obtained after the transient adaptation processes the quasi-stationary structure of network corresponds to the topology of scale-free network, specifically the distribution of nodes weights corresponds to power law. This suggests that the studied network model can be used to explain the effects observed in the real networks which are characterized by the scale-free topology. In particular, obtained results can be applied for explanation of evolution of simple neuronal networks to complex hierarchical structure of real neuronal network of the brain. We have obtained conditions of establishment different topology of networks in dependence of coupling parameters.

10063-47, Session PSun

Influence of earlobe thickness on near infrared spectroscopy

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Near-infrared spectroscopy has been recognized as a potential technology for noninvasive blood glucose sensing. However, the detected spectral signal is unstable mainly because of (1) the weak light absorption of glucose itself with NIR range, ?2?the influence of temperature and individual differences of biotissue. Our previous results demonstrated that the synergistic effect of both transmittance and reflectance could enhance the strength of the detection signal. In this talk, we design a set of experiments to analyze the effect of earlobe thickness on Near Infrared spectroscopic measurement by using home-made optical fiber probe within the wavelength of 1000-1600nm. Firstly, the diffused transmittance spectra and diffused reflectance spectra of the 2% Intralipid have been collected under different optaical path lengths. And then we obtain the spectra of the earlobes from different volunteers by the same way. The experimental results showed that the light intensity of diffused transmittance decease, and the light intensity of diffused reflectance increase with the increase of the thickness.

10063-48, Session PSun

Experimental study on the influence of the contact pressure to transmittance and reflectance spectra by Near Infrared Spectroscopy

Si Li, Tianpei Wang, Lin Li, Jiajia Liu, Jingying Jiang, Kexin Xu, Tianjin Univ. (China)

Near Infrared Spectroscopy (NIRS)technology has been recognized as one of the most promising non-invasive blood glucose measurement methods due to its convenience, high efficiency, noninvasiveness, and real-time monitoring. We built a system to measure transmittance and reflectance with NIR simultaneously. And contact measuring method has been performed in order to reduce the influence of specular reflectance of the measured skin tissue. However, in this way, the optical probe could press the skin tissue and make it distorted, which makes the internal structure and the constituent distribution of tissue changed and further the tissue optical parameter changed. This could eventually change the distribution of transmittance spectra and reflectance spectra. In this talk, we collected the transmittance spectra and the diffused reflectance spectra of earlobe within the wavelength of 900-1700nm under the different contact pressures. The results show that before the probe contacts with the earlobe, the specular reflectance reduces with the distance between the probe and the earlobe, after the probe contacts with the earlobe, the diffused reflectance spectra decrease and the diffused transmittance spectra increase with the increase of the contact pressure. At the same contact state, in the initial stage of the earlobe after compression, diffused transmittance and diffused reflectance spectra fluctuate dramatically with the time course. After a certain period of stabilization, the spectral fluctuations tend to be steady.

10063-49, Session PSun

Artifact removal from EEG data with empirical mode decomposition

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In the present report we introduce a new method for removing different artifacts from EEG signals. As experimental data we consider EEG signals of healthy volunteers with standard physiological probes. Artifacts are various oscillations on EEG caused by non-brain activity: other physiological signals or external electromagnetic fields. Artifacts usually have considerable amplitude and cover a wide frequency band on EEG signals so systems for artifact removal are important in all kind of EEG research.

Method proposed in the report is based on empirical mode decomposition that decompose initial EEG signal into the set of amplitude-modulated components – empirical modes. Procedure of empirical mode decomposition includes several steps: finding all extrema (minima and maxima) on signal, construction of two corresponding envelopes, calculation of low-frequency trend of signal as mean value of two envelopes, extraction of empirical mode as residue of the initial signal and trend. Each empirical mode includes its own set of frequency components derived from the initial EEG signal and thus possesses its own set of oscillatory patterns. Some empirical modes include information about different EEG components while other modes mostly consist of artifacts. The essence of proposed method is to delete some empirical modes that include artifacts and to reconstruct EEG signal by summarizing the rest empirical modes.

The developed method is tested on EEG signals of 15 volunteers. High efficiency in removing of artifacts of different nature from EEG is shown.

10063-50, Session PSun

Common-path biodynamic imaging for dynamic fluctuation spectroscopy of 3D living tissue

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We present a common-path optical interferometry system for biodynamic imaging of living three-dimensional tissue. Biodynamic imaging is a novel 3D optical imaging technology based on short-coherence digital holography that measures intracellular motions of cells inside their natural microenvironments. Intracellular dynamics are captured through fluctuation spectroscopy from the Doppler beats of the intracellular components that produce a fluctuating intensity encoding the individual Doppler frequencies. Dynamic intracellular mechanisms that produce fluctuation frequencies within our detection bandwidth include organelle transport, membrane undulations, cytoskeletal restructuring, strain at cellular adhesions, cytokinesis, mitosis, exo- and endo-cytosis among others. In the common-path configuration, specular reflection from the bottom of a 96-well plate is used as the reference arm and passes through the same optical elements as the signal arm, instead of introducing a standalone reference arm which is typical of a Mach-Zehnder setup. The difference in the optical path lengths of the two arms is insensitive to vibrations or temperature fluctuations, leading to stable holograms captured by the camera. Biological tissues such as tumor spheroids and ex vivo biopsies are used as targets, and the backscattered light is collected as signal. Drugs are applied to tumors and their effects are evaluated by identifying biomarkers that capture intracellular dynamics from the reconstructed holograms. By adjusting the delay on the reference arm, information from different depths of the tumors can be extracted, enabling the deep-tissue measurement of the responses of the drugs.

10063-51, Session PSun

Numerical and analytical investigation of the chimera state excitation conditions in the Kuramoto-Sakaguchi oscillator network

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Nowadays methods of nonlinear dynamics are widely used in such field of modern science as neuroscience. Special interest in their application is induced in the area of brain research. Currently, many general effects of brain activity are well-described in terms of complex networks consisting of nonlinear oscillators. Such success in the modeling of brain dynamics has given a significant opportunity to reveal the effects that occur due to the interaction between large number of nonlinear elements grouped in networks with various types of connection topology. Recently discovered phenomenon known as “chimera state” especially arouses the interest of the scientific community. This effect consists in the simultaneous coexistence of the coherent phase-locked oscillators and incoherent oscillators in the complex network.

In the current letter we have carried both the numerical and the theoretical

treatment to find the conditions of the chimera state excitation in the Kuramoto-Sakaguchi (KS) phase oscillator network with nonlocal coupling. Theoretical research has been based on the Ott-Antonsen approach that allows to analyze the behavior of the complex network of coupled oscillators in terms of the probability density function assuming the infinite number of interacting oscillators. We have compared the results obtained within the analytical model with the results of KS network numerical calculation in the framework of the Runge-Kutta iteration scheme. In both cases we find the similar phase distributions of KS phase oscillators corresponding to the appearance of the chimera state in KS network.

10063-52, Session PSun

The control of the frequency of the sub-terahertz source on the semiconductor superlattices for biophysical applications with use the change of the doping density

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In the present report we consider the control method of the frequency characteristics of the semiconductor superlattice with change the doping density. The devices on the semiconductor superlattice are perspective for biophysical applications how sub-terahertz source. Semiconductor superlattice are complex structures consistent of several think layer of different semiconductor materials. The application of the electric field lead to forming electron domains, that transport through superlattice excite the current oscillation with sub-terahertz frequency. Different influence can sufficiently change electron dynamic and current oscillation, for example magnetic field, temperature, interminiband tunneling and other. In this report we show that frequency of the current oscillations in semiconductor superlattice is dependent on doping density close by emitter. The growth of the doping density lead to the increase of the value of voltage wherein start current oscillation. At the same time the frequency value for fixed voltage is increase, too. For lesser value of the doping density value of voltage wherein start current oscillation decrease, but maximum value of frequency higher than for normal doping density close by emitter. Thus we can control the frequency characteristics of the semiconductor superlattice with changing of the doping density. More than that, we can change frequency characteristics without create new device, it is enough growth new layer of structure with different doping density into existent superlattice.

10063-53, Session PSun

Numerical analysis of the chimera states in the multilayered network model

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Currently, the world scientific community demonstrates the great interest in the study of chimera states, arising in the ensembles of nonlinear oscillators of the different nature.Such states, characterized by the coexistence of thegroups of coherent and incoherent elements in the networks of coupled oscillators, were observed in the variety of the network, characterized by the different properties of the nodes and topology. At the same time, the majority of previous researches were limited by the analysis of the chimera states in single network and did not take into account the interaction between them.

According to this, in the current letter we study the interaction between the ensembles of nonlocaly coupled oscillators, arranged in the multilayer network. We have shown that the fully identical layers, demonstrated individually different chimera due to the initial mismatch, come to the identical chimera state with the increase of inter-layer coupling. Within the multilayer model we also consider the case, when the one layer demonstrates chimera state, while another layer exhibits coherent or incoherent dynamics. It has been shown that the interactions chimera-coherent state and chimera-incoherent state leads to the both excitation of chimera as from the ensemble of fully coherent or incoherent oscillators, and suppression of initially stable chimera state

10063-55, Session PSun

Porphyrinic probes for targeted attack of cancer cells transferrin receptors

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Transferrin receptors (TfR) are expressed on the tumor cells in large quantities due to high needs of iron in tumor development whereas in normal cells they generally present in low level. The targeted binding of probe molecules to receptors of cancerous cells can disrupt the operation or destroy the receptors, or targeted agent can be captured by a cancer cells, which ultimately leads to the destruction of cancer cells.

For targeted attack of transferrin receptor we was constructed a new anticancer agent-probe consisting of modified transferrin (Tf) and a cationic porphyrin (P) (probe transferrin-porphyrin [Tf-P]), which is activated via photodynamic action. After capturing of the probe [Tf-P] by transferrin receptor these complex [TfR-Tf-P] is uptakes by the cancerous cell with formation of the vesicles in the cells. Then pH inside of vesicles decreases due to operation of protonic ion pumps, causing transferrin released the iron ions, and a porphyrin desorbed from transferrin and penetrates into cell cytoplasm. At photodynamic action the porphyrins or probes [Tf-P] violate of cells operation (by formation of singlet oxygen) that causes damage in cell structures and destruction of cancer cells. We have investigated the effect of probes [Tf-P] (with various cationic porphyrins) on the culture of human breast cancer cells MCSF7 in a dark and photodynamic modes. The most active probes and the degree of damage to the culture of cancer cells have been identified.

10063-56, Session PSun

Comparative study of the optical properties of colon mucosa and colon precancerous polyps between 400 and 1000 nm

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Optical properties of biological tissues are unique and may be used for tissue identification, to discriminate between different tissues or even to identify pathologies. Early stage colon cancer consists on polyp formation in the most inside layer of the colon tube – the mucosa. The identification of different optical properties between the healthy colon mucosa and colon cancer polyps might be the basis for developing a noninvasive and early stage diagnosis method using optical methods. Since most of the biomedical optics techniques applied in clinical practice use light within the visible and near infrared wavelength range, we have estimated the optical properties of colon mucosa and cancer polyps in this range. The estimated data shows different values between the two types of tissues. Such data can now be used for instance to model and optimize an optical clearing treatment that will increase tumor transparency for early cancer diagnostics or treatment procedures. Due to the difference in the scattering properties of the two tissues, a noninvasive and early stage cancer detection method based on light reflectance can now be investigated. Its application in colon and also for study of upper airway tubes can be of major interest in clinical practice, both for clinician and patient.

10063-57, Session PSun

Preparation of novel in vivo skin optical clearing solution: theoretical-prediction-based experimental demonstration

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Tissue optical clearing technique has been showing tremendous potential in optimizing performance of various optical imaging modalities. Herein, we proposed two novel and powerful in vivo skin optical clearing solutions (SOCS), termed PST and PSPG, based on the theoretical prediction performed via molecular dynamics (MD) simulation. Optical coherence tomography angiography was applied to quantify the two SOCS-induced skin optical clearing efficacy based on imaging performance optimization, changes of skin optical properties and refractive index mismatching extent, as well as permeability rate. These results were further compared with some SOCS reported before. This study demonstrated that some more novel SOCS can be developed by means of additionally adding or replacing the similar category substance in pre-existing SOCS with some much better optical clearing reagents screed out by MD simulation, and it will provide useful principles for developing more novel and powerful SOCS.

10063-59, Session PSun

Acoustic radiation force enhanced displacement of gold nanoparticles (GNPs) monitored by using OCT

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In this study, we present a technique to image the enhanced particle displacement generated using an acoustic radiation force (ARF) excitation source. A MEMS-VCSEL swept source Optical Coherence Tomography (SS-OCT) system with a center wavelength of 1310nm, a bandwidth of 100nm, and an A-scan rate of 100 kHz was used to detect gold nanoparticle (70 nm in diameter) displacement induced by the ARF of a ultrasound (US) beam. B-mode, M-B mode, 3D and Speckle Variance (SV) images were acquired before and after the US beam was on. Differential OCT speckle variance images with and without the ARF were used to estimate the microscopic enhancement of nanoparticle displacement generated by the ARF. Different concentrations of collagen (3% ,6% and 10%) and a tissue engineered cellular construct based on MCF7 breast cancer cells embedded in a collagen matrix were used in this study. Enhanced displacement of the GNPs was calculated in both the axial and lateral directions at locations close to the ultrasound focal point, associated with longitudinal waves and shear waves, respectively. We selected a region of interest (ROI) close to the ultrasound focus to calculate the displacement map (parametric map) and analyze the enhanced displacement (axial or lateral). The displacement associated with the enhanced transport decreased as the collagen concentration increased. Using the SV-OCT technique, we are able to visualize and characterize the acoustic radiation force assisted enhanced displacement of gold nanoparticles (GNPs) in collagen matrices.

10063-16, Session 5

Optical diagnosis of cervical cancer by boosting algorithm

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In this contribution, we report the application of ensemble based learning methodologies as diagnostic algorithms for optical diagnosis of cancer. The classification was done using Random Forest and Bag of decision tree model and the results were compared to those obtained with an independent feature extractors like linear discriminant analysis (LDA) and principal component analysis (PCA). The performance and efficacy of these methodology using ensemble has higher specificity and sensitivity while being compared with other machine learning tools.

10063-17, Session 5

Characterizing microstructural features of biomedical samples by statistical analysis of Mueller matrix images

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As one of the salient features of light, polarization contains abundant structural and optical information of media. Recently, as a comprehensive description of polarization property, the Mueller matrix polarimetry has been applied to various biomedical studies such as cancerous tissues detections. In previous works, it has been found that the structural information encoded in the 2D Mueller matrix images can be presented by other transformed parameters with more explicit relationship to certain microstructural features. In this paper, we present a statistical analyzing method to transform the 2D Mueller matrix images into frequency distribution histograms (FDHs) and their central moments to reveal the dominant structural features of samples quantitatively. Moreover, we obtain a new set of FDH based parameters, which can be expressed as analytical functions of the central moments of 16 Mueller matrix elements. Both the experimental and Monte Carlo simulated results demonstrate that the

parameters based on the statistical analysis of Mueller matrix elements have simple relationships to the dominant microstructural properties of biomedical samples, including the density and orientation of fibrous structures, the depolarization power, diattenuation and absorption abilities. In addition, we apply the FDH based Mueller matrix parameters to several human pathological tissue samples. Preliminary imaging results indicate that the parameters can quantitatively reflect the changes of fibrous tissues accompanying the pathological processes. It is shown in this paper that the statistical analysis of 2D images of Mueller matrix elements may provide quantitative or semi-quantitative criteria for biomedical diagnosis.

10063-18, Session 5

SHG imaging of strain-dependent collagen architecture as a model for bone growth acceleration after periosteal resection

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Previous studies have shown that bone growth acceleration can occur in many animal species after periosteal resection (removal of a strip of periosteum) with minimum morbidity. This has numerous clinical applications, including treatment of limb length differences. Here we use Second Harmonic Generation (SHG) imaging microscopy to evaluate changes in collagen architecture reflective of the different strains the periosteum may encounter during bone growth. Specifically, we image rabbit tibial periosteum strips at -20%, 0%, 5%, and 10% strains. We first quantify these changes using the SHG creation ratio (Forward/Backward) or the initially emitted SHG directionality to provide information on the fibril level of assembly. The in situ (i.e. physiological) strain had the highest creation ratio compared to the non-in situ strains of -20%, 5%, and 10%, which were shown to be significantly different via RCBD statistical analysis. These trends are consistent with SHG phasematching considerations, where more organized fibrils/fibers result in primarily forward emitted components, which here is the physiological strain. We further use the relative SHG conversion efficiency to assess the tissue structure under strain, where this results from the combination of collagen concentration and organization. The 0% strain SHG conversion efficiency was significantly higher than all other strains, where this is expected as the fibers have the highest local density and organization, and is consistent with the emission directionality results. Importantly, due to the underlying physical process, the label-free SHG imaging modality can non-invasively monitor the effect of treatments for bone growth and other orthopedic disorders.

10063-19, Session 5

Visualization of extracellular vesicles using label-free multimodal multiphoton imaging *(Invited Paper)*

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Extracellular vesicles (EVs) containing nucleic acids, proteins, and lipids play key roles in intercellular communication. Real-time spatiotemporal analysis of these nanometer-scale EVs is essential to understand EV-mediated communication in tumor development and progression. Previous studies have developed various fluorescent labeling methods to enable visualization and tracking of EVs, but strictly non-perturbative and label-free techniques have been lacking, mainly due to the low sensitivity and specificity of the intrinsic contrast of small EVs against their surrounding background environments. Here we propose to utilize multimodal multiphoton imaging to provide simultaneous, co-registered structural and functional images of EVs in untreated samples. The heterogeneous populations of EVs could be identified via unique optical signatures acquired from multiple modalities, including third harmonic generation to delineate structural interfaces and two/three-photon to quantify FAD and NADH concentrations. To examine the accuracy of these intrinsic optical signatures for identifying EVs, we imaged fractionated EVs that were labeled with membrane-specific fluorescent markers and observed spatial co-registration between the fluorescent markers and the intrinsic optical signatures. These results suggest that multimodal multiphoton imaging is capable of direct label-free visualization of EVs with high sensitivity and specificity, and has strong potential to enable unprecedented label-free exploration of EV dynamics in living organisms in future studies.

10063-58, Session 5

Recent advances in theranostics of circulating tumor cells in vivo using magnetic contrast agents

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Clinical application of circulating tumor cells (CTCs) for monitoring cancer progression has been substantially developed in recent years. However, clinically-relevant technology for killing CTCs as potential source of deadly cancer metastasis has not been well defined. Here we summarize our advances in CTC theranostics in vivo using photoacoustic (PA) flow cytometry (PAFC) platform with magnetic nanoparticles (MNPs) as PA, photothermal (PT) and magnetic contrast agents. PAFC demonstrate ultrasensitive counting CTCs (the sensitivity threshold >100 times higher than in the existing assays) in relatively deep (1-3 cm) blood vessels with fast flow (up to 5-20 cm per a sec) using safe for human laser parameters. Low-toxic MNPs conjugated with ligands (e.g., folate, antibodies) specific to CTC receptors provides CTC labeling for (1) molecular CTC targeting and (2) magnetic capturing (trapping) with magnet attached to the skin near detection area. Captured CTCs can be destroyed by laser resulting in disappearance of CTC-related PA signals that serves as an indicator of therapy efficiency. The properties of novel bioinspired MNPs and hybrids of MNPs including nanocomposite microcapsules (NMs) containing magnetite nanoparticles with and without indocyanine green (ICG) were tested in vitro (e.g., toxicity, PA contrast, spectra, molecular specificity and therapeutic effects) and in vivo (detection of intravenously injected mimic CTCs in mouse models). In our preclinical studies, we demonstrated counting of real CTCs and magnetic manipulations of CTCs in circulation of tumor (breast)-bearing mice. Overall, our findings show that PAFC and NMs with MNPs as PA, PT and magnetic agents provides unique multifunctional assessment of CTCs, which is unachievable with the existing techniques, and has potential for the early theranostics of CTCs for prevention of metastasis in humans.

10063-20, Session 6

Skull optical clearing for enhancing cortical microcirculation imaging with high contrast and resolution *(Invited Paper)*

Dan Zhu, Huazhong Univ. of Science and Technology (China)

The tissue optical clearing technique could significantly enhance the biomedical optical imaging depth, but current investigations are mainly limited to in vitro studies. In vivo tissue optical clearing method should be enough rapid, transparent and safe, which makes it more difficult, especially, for hard tissue. During the past years, we developed skull optical clearing methods for in vivo cortical imaging. This presentation will report recent progress in skull optical clearing method, including their efficacy, safety, and applications. The skull optical clearing method is proved to be effective for adult mice ages in different month and permit various imaging techniques to monitor cortical blood flow, blood oxygen, and vascular with high resolution and contrast, not only for local cortex, but also for whole cortex. The long-term and short-term observation show that there is no obvious effect on cortical vascular function when laser speckle contrast imaging and hyperspectral imaging are used to repeatedly image the cortical blood flow, blood oxygen. Finally, we will demonstrate some applications for physiological or pathological situation, including monitoring the anoxia, drug-induced cortical response, et al.

10063-21, Session 6

Optical clearing of melanoma in-vivo: characterization by optical coherence tomography

Valentin Demidov, Univ. of Toronto (Canada); Layla Pires, Univ. of Toronto (Canada) and Univ. de São Paulo (Brazil); I. Alex Vitkin, Univ. of Toronto (Canada) and Princess Margaret Cancer Ctr., Univ. Health Network (Canada); Vanderlei S. Bagnato, Cristina Kurachi, Univ. de São Paulo (Brazil); Brian C. Wilson, Univ. of Toronto (Canada) and Princess Margaret Cancer Ctr., Univ. Health Network (Canada)

We report on quantitative assessment of effect of a topically-applied optical clearing agent (OCA) on the microvascular network imaging of melanoma tumors in vivo with optical coherence tomography (OCT). Being the most aggressive type of skin cancer melanoma has a significant risk of fatality. During the phase of vertical growth it develops dense neovascularization that has been correlated with poor prognosis and overall survival, tumor ulceration and increased rate of recurrence. Melanin pigmentation results in a high visible-light absorption, so that optical imaging techniques are limited to probing only near the tumor surface, which is inadequate to evaluate the microvascular density. A potential solution lies with OCT, a non-invasive scanning technique that enables volumetric depth-resolved cross-sectional imaging of subsurface tissue microstructure.

Speckle variance OCT imaging of microvasculature was performed before and up to 4 hours after OCA application. 3D datasets of tumor-associated and normal vasculatures were presented as two-dimensional depth-encoded microvascular maps and compared for several time points after OCA application. The clearing effect was quantified using spatial texture analysis of OCT image speckle patterns.

OCT was able to image the microvasculature in the pigmented melanoma tissue with 15?m spatial resolution up to a depth ~300 μm without the use of OCA; improved contrast-resolution was achieved with optical clearing to a depth of ~750 μm in tumor. These findings are relevant to potential clinical applications in melanoma, such as assessing prognosis and treatment responses. Optical clearing may also facilitate the use of light-based treatments such as photodynamic therapy.

10063-22, Session 7

Prototype of an opto-capacitive probe for non-invasive sensing cerebrospinal fluid circulation *(Invited Paper)*

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In brain studies, the function of the cerebrospinal fluid (CSF) awakes growing interest, particularly related to studies of the so-called glymphatic system in the brain, which is connected with the complex system of lymphatic vessels responsible for cleansing the tissues. The CSF is a clear, colourless liquid including water (H2O) approximately with a concentration of 99 %. In addition, it contains electrolytes, amino acids, glucose, and other small molecules found in plasma. The CSF acts as a cushion behind the skull, providing basic mechanical as well as immunological protection to the brain. Disturbances of the CSF circulation have been linked to several brain related medical disorders, such as dementia.

Our goal is to develop an in vivo method for the non-invasive measurement of cerebral blood flow and CSF circulation by exploiting optical and capacitive sensing techniques simultaneously. We introduce a prototype of a wearable probe that is aimed to be used for long-term brain monitoring purposes, especially focusing on studies of the glymphatic system.
In this method, changes in cerebral blood flow, particularly oxy- and deoxyhaemoglobin, are measured simultaneously and analysed with the response gathered by the capacitive sensor in order to distinct the dynamics of the CSF circulation behind the skull. Presented prototype probe is tested by measuring liquid flows inside phantoms mimicking the CSF circulation.

10063-23, Session 7

Intrinsic fluorescence of protein in turbid media using empirical relation based on Monte Carlo lookup table

Einstein Gnanatheepam, Prakasa Rao Aruna, Ganesan Singaravelu, Anna Univ., Chennai (India)

Fluorescence of Protein has been widely used in diagnostic oncology for characterizing cellular metabolism. However, the intensity of fluorescence emission is affected due to the absorbers and scatterers in tissue, which may lead to error in estimating exact protein content in tissue. Extraction of intrinsic fluorescence from measured fluorescence has been achieved by different methods. Among them, Monte Carlo based method yields the highest accuracy for extracting intrinsic fluorescence. In this work, we have attempted to generate a lookup table for Monte Carlo simulation of fluorescence emission by protein. Furthermore, we fitted the generated lookup table using an empirical relation. The empirical relation between measured and intrinsic fluorescence is validated using tissue phantom experiments. The proposed relation can be used for estimating intrinsic fluorescence of protein for real-time diagnostic applications and thereby improving the clinical interpretation of fluorescence spectroscopic data.

10063-24, Session 7

Mueller matrix microscopy for label-free histopathology examinations

Donghong Lv, Tsinghua Univ. (China); Honghui He, Graduate School at Shenzhen, Tsinghua Univ. (China); Jialing Zhou, Hui Ma, Tsinghua Univ. (China)

In clinical medicine, a pathologist often needs to examine cells or thin slices of tissues to identify abnormalities that are markers or precursors of diseases. Various chemical and immunohistochemical staining techniques have been developed to selectively label certain components to bring up the contrasts of specific microstructures. It is well known that a Mueller matrix contains rich information on the microstructure and optical properties of a sample. Using proper data analysis techniques, Mueller matrix images can also be transformed into new polarization parameters sensitive only to specific microstructural features. These new polarization parameters can selectively enhance the contrast of specific features in images of unstained pathological slide to help identify abnormalities. In recent studies, we set up a modulus design Mueller matrix microscope by adding polarization optics components into the optical path of a commercial transmission microscope. We take multiple measurements of the unstained pathological slide at different polar and azimuth angles, then derive an intrinsic Mueller matrix (IMM) which represents only the microstructural characters of the sample without the interference by the sample orientation. Such orientation-independent IMM images preserve to the maximum extent the pathological information of the tissue samples. Using Mueller matrix decomposition and transformation techniques, we demonstrate in preliminary tests that we are able to selectively enhance different characteristic features in different cancer tissues. With the fast advances in big-data analysis techniques, it is expected that label-free Mueller matrix microscopy is a potentially powerful tool for the histopathologists to identify characteristic features in complex tissue samples.

10063-25, Session 7

Quantifying time-of-flight-resolved temporal dynamics of optical field scattered from the turbid media using interferometric near-infrared spectroscopy (iNIRS)

Dawid Borycki, Oybek Kholiqov, Wenjun Zhou, Vivek J. Srinivasan, Univ. of California, Davis (United States)

Sensing and imaging methods based on the dynamic scattering of coherent light, including laser speckle, laser Doppler, and diffuse correlation spectroscopy quantify scatterer motion using light intensity (speckle) fluctuations. The underlying optical field autocorrelation (OFA), rather than being measured directly, is typically inferred from the intensity autocorrelation (IA) through the Siegert relationship, by assuming that the scattered field obeys Gaussian statistics. In this work, we demonstrate interferometric near-infrared spectroscopy (iNIRS) for measurement of time-of-flight (TOF) resolved field and intensity autocorrelations in fluid tissue phantoms and in vivo. In phantoms, we find a breakdown of the Siegert relationship for short times-of-flight due to a contribution from static paths whose optical field does not decorrelate over experimental time scales, and demonstrate that eliminating such paths by polarization gating restores the validity of the Siegert relationship.

Inspired by these results, we developed a method, called correlation gating, for separating the OFA into static and dynamic components. Correlation gating enables more precise quantification of tissue dynamics. To prove this, we show that iNIRS and correlation gating can be applied to measure cerebral hemodynamics of the nude mouse in vivo using dynamically scattered (ergodic) paths and not static (non-ergodic) paths, which may not be impacted by blood. More generally, correlation gating, in conjunction with TOF resolution, enables more precise separation of diffuse and non-diffusive contributions to OFA than is possible with TOF resolution alone. Finally, we show that direct measurements of OFA are statistically more efficient than indirect measurements based on IA.

10063-26, Session 7

Noise sources in Raman spectroscopy of biological objects

Janusz M. Smulko, Maciej S. Wróbel, Gdansk Univ. of Technology (Poland)

We present an overview of noise sources deteriorating quality of recorded biological Raman spectra and ability of determining the specimen composition. Raman spectroscopy is a widely used method to investigate chemical molecules in biological specimens but the acquired Raman spectra exhibit intense additive noise components or drifts. Therefore we have to apply expensive or bulky measurement setups to limit their inherent noise or to apply additional signal processing methods to reduce random components after recording the spectra. We present noise sources generated in detectors of Raman scattered photon stream. We consider the methods of background noise reduction by increasing averaging time when the background noise comprises of white noise and 1/f-type noise components. Next, we scrutinize efficiency of popular processing methods reducing the background noise in the acquired spectra (e.g., Savitzky-Golay filtering, polynomial approximation, denoising by empirical mode decomposition). Moreover, we consider how the background noise reduces accuracy of chemical compounds estimation using Raman spectra and prediction model based on linear (e.g., Principal Component Analysis) or nonlinear (e.g., Support Vector Machine) methods. Finally, we give some remarks about efficiency of applying synchronous detection to reduce background noise when we record consecutively a series of Raman spectra at relatively short averaging time and the reference signal modulating these spectra. The exemplary Raman spectra were collected using two excitation lasers of the wavelengths 785 nm or 830 nm.

10063-27, Session 7

Diagnostics of oral lichen planus based on analysis of volatile organic compounds in saliva

Yury V. Kistenev, National Research Tomsk State Univ. (Russian Federation); Olga V. Baydik, Siberian State Medical Univ. (Russian Federation); Alexander V. Shapovalov, Alexey V. Borisov, National Research Tomsk State Univ. (Russian Federation); Maria A. Titarenko, Siberian State Medical Univ. (Russian Federation)

The ability of diagnostics of oral lichen planus (OLP) based on analysis of volatile organic compounds in saliva using the technique of laser photoacoustic spectroscopy (PAS) is discussed.

Cytological studies showed the number of cells with normal nucleus and cytoplasm structure in the control group was 61.4%, while in patients with erosive and reticular / papular forms of OLP this parameter decreased to 34.7% and 54.0%, respectively. The erosive form of OLP 0 and 4th types of destruction of epithelial cells were observed almost equally, the content of OLP cells was significantly lower compared to the control.

Laser PAS showed the levels of N2O in all patients with OLP were significantly decreased in comparison with the control group. The levels of NH3 and NO2 in patients with the erosive form of OLP were significantly decreased when compared to the control group the group of reticular / papular form of OLP.

Determination of N2O and NO2 in the saliva evaporation by LPAS in conjunction with exfoliative cytology can be an additional method in diagnosis and prognosis of OLP.

10063-28, Session 8

Characterization of relationship between OMAG signal and blood flow *(Invited Paper)*

Woo June Choi, Wan Qin, Chieh-Li Chen, Jingang Wang, Qinqin Zhang, Ruikang K. Wang, Univ. of Washington (United States)

Optical microangiography (OMAG) is a powerful optical angiographic tool to visualize micro-vascular flow in vivo. Despite numerous demonstrations for the past several years of the qualitative relationship between OMAG and flow, no convincing quantitative relationship has been proven. In this paper, we attempt to quantitatively correlate the OMAG signal with flow. Specifically, we develop a simplified analytical model of the complex OMAG, suggesting that the OMAG signal is a product of the number of particles in an imaging voxel and the decorrelation of OCT (optical coherence tomography) signal, determined by flow velocity, interframe time interval, and wavelength of the light source. Numerical simulation with the proposed model reveals that if the OCT amplitudes are correlated, the OMAG signal is related to a total number of particles across the imaging voxel cross-section per unit time (flux); otherwise it would be saturated but its strength is proportional to the number of particles in the imaging voxel (concentration). The relationship is validated using microfluidic flow phantoms with various preset flow metrics. This work suggests OMAG is a promising quantitative tool for the assessment of vascular flow.

10063-29, Session 8

Spectral contrast-enhanced optical coherence tomography for improved detection of tumor microvasculature and functional imaging of lymphatic drainage

Elliott D. SoRelle, Orly Liba, Debasish Sen, Adam de la Zerda, Stanford Univ. (United States)

Optical Coherence Tomography (OCT) is well-suited to study in vivo dynamics of blood circulation and lymphatic flow because of the technique's combination of rapid image acquisition, micron spatial resolution, and penetration depth in turbid tissues. However, OCT has been historically constrained by a dearth of contrast agents that are readily distinguished from the strong scattering intrinsic to biological tissues. In this study, we demonstrate large gold nanorods (LGNRs) as optimized contrast agents for OCT. LGNRs produce 32-fold greater backscattering than GNRs previously tested for contrast-enhanced OCT. Furthermore, LGNRs exhibit 110-fold stronger spectral signal than conventional GNRs when coupled with custom spectral detection algorithms. This signal enhancement enables picomolar OCT detection sensitivity in vivo and single-particle detection against optically-clear backgrounds. Moreover, the ability to synthesize LGNRs with tunable spectral peaks provides a viable platform for multiplexed imaging studies. To explore the advantages of LGNRs as OCT contrast agents, we implemented them for noninvasive 3D imaging of tumor blood supply and active lymphatic drainage in mice. Spectral detection of LGNRs enabled 100% improvement in imaging depth for detecting microvasculature (vessels ~ 20 ?m in diameter) in U87MG glioblastoma xenografts in mice pinnae. We also demonstrated our approach's ability to map the spatial dependence of lymph drainage and flow directionality within lymphatic capillaries. Using LGNRs with distinct spectra, we further identified the functional states of individual lymphatic valves in vivo. Thus, this approach provides a powerful new platform for functional imaging that may be extended for future molecular imaging studies with OCT.

10063-30, Session 8

Quantification of flow rate from optical coherence tomography based angiography (OCTA) signal

Shaozhen Song, Jingjiang Xu, Qinqin Zhang, Yuandong Li, Univ. of Washington (United States)

The quantification of blood flow rate in OCT imaging has been an outstanding problem to date. Although a wide-span of analysis on OCT angiography signal and Doppler OCT signal were conducted, it's difficult to achieve absolute quantification of in vivo blood flow velocity. This study shows the evidence from theoretical aspects and experimental results that scan repetition rate has been the fundamental limitation of the flowmetry dynamic range. Enabled by ultra-fast, phase stabilized swept-source OCT (SS-OCT) system, ultra-high frame rate OCTA scan protocol is introduced. From the phantom study, the dynamic range of OCTA is proved to be significantly elevated. With the high frame rate of 8 kHz, the highest flow velocity without OCTA signal saturation is estimated to be ~3.5 mm/s, while the sensitivity to very low speed blood flow is also maintained, which satisfies the demand of major applications of OCTA flowmetry. The optical microangiography (OMAG) signal is also compared with the Doppler OCT signal, to show the advantage of direction-independence of the OCTA flowmetry. The proposed approach is tested by mouse brain blood vessel imaging, where the flow velocity in capillaries and relatively large blood vessels are quantified and mapped. It is hoped this approach can become useful as a clinical tool for visualizing blood vessels with reliable absolute flow velocity information.

10064-1, Session 1

Photoacoustic tomography: deep imaging beyond the optical diffusion limit *(Invited Paper)*

Lihong V. Wang, Washington Univ. in St. Louis (United States)

No Abstract Available.

10064-2, Session 1

Towards genetically encoded Indicators for photoacoustic detection of neuronal activity *(Invited Paper)*

Robert E. Campbell, Univ. of Alberta (Canada)

Interest in genetically encoded indicators of neuronal activity has exploded over the past decade as the tremendous practical utility of these tools has become apparent to the growing number of neuroscientists racing to decipher the inner workings of the brain. While the current generation of visibly fluorescent calcium ion indicators is highly optimized for superficial imaging of neuronal activity, there is growing demand for tools that could enable researchers to visualize activity deeper into the brain. In response to these demands, my research group is working to develop a first generation of genetically encoded indicators with excitation in the near-infrared optical window where tissue is most transparent to light. In this seminar I will present some of our most recent efforts to engineer this next generation of improved far-red and near-infrared calcium ion indicators optimized for both fluorescence and photoacoustic imaging.

10064-3, Session 1

Engineering of bacterial phytochromes for in vivo imaging *(Invited Paper)*

Vladislav Verkhusha, Daria M. Shcherbakova, Andrii A. Kaberniuk, Mikhail Baloban, Albert Einstein College of Medicine (United States)

Genetically encoded probes with absorbance and fluorescence spectra within a near-infrared tissue transparency window are preferable for deep-tissue imaging. On the basis of bacterial phytochromes we engineered several types of near-infrared absorbing probes for photoacoustic tomography and fluorescent probes for purely optical imaging. They can be used as protein and cell labels and as building blocks for biosensors. The probes enabled imaging of tumors and metastases, protein-protein interactions, RNA visualization, detection of apoptosis, cellular metabolites, signaling pathways and cell proliferation. The developed probes allow non-invasive visualization of biological processes across scales, from super-resolution microscopy to tissue and whole-body animal imaging.

10064-4, Session 1

Multiscale photoacoustic tomography of GCaMP6-expressing mouse brains

Ruiying Zhang, Lei Li, Bin Rao, Washington Univ. in St. Louis (United States); Junjie Yao, Duke Univ. (United States); Min-yu Sun, Steven Mennerick, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Photoacoustic (PA) tomography has been demonstrated as a powerful brain imaging modality capable of providing high-resolution images with optical absorption at depths far beyond the optical diffusion limit. Previous endeavors in PA brain studies have focused on measuring hemodynamic parameters, which are relatively slow indicators of neural activities through neurovascular coupling. Intracellular calcium signals, on the other hand, are faster indicators of neural activities than hemodynamic signals. Here we apply two implementations of photoacoustic tomography, photoacoustic microscopy (PAM) and photoacoustic computed tomography (PACT), for neuronal calcium imaging in mouse brain models, using the genetically encoded calcium indicator GCaMP6. First, we acquired ex vivo PAM and PACT images of GCaMP6 mouse brain tissues, showing robust PA signals. Then, with the state of the art PACT system, we unambiguously imaged wide-field neuronal calcium signals, induced by high-potassium perfusion, in a coronal section of the GCaMP mouse brain slice. Moreover, by utilizing diffusive photons, we demonstrated that neural responses can be recorded by PACT, even from brain slices 2mm beneath a thick scattering medium. Finally, we recorded increased PA signals in GCaMP6 mouse brain tissue in vivo, in response to hindpaw electrical stimulations. With optimized genetically encoded neural activity indicators, multiscale PAT holds great promise for high resolution and deep penetration imaging of neural activities in vivo.

10064-5, Session 1

In vivo deep brain imaging of rats using photoacoustic computed tomography

Li Lin, Lei Li, Liren Zhu, Peng Hu, Washington Univ. in St. Louis (United States); Lihong V. Wang, Washington Univ. in St. Louis (United States) and California Institute of Technology (United States)

The brain has been likened to a great stretch of unknown territory consisting of a number of unexplored continents. Small animal brain imaging plays an important role charting that territory. By using 1064 nm illumination from the side, we imaged the full coronal depth of rat brains in vivo. The experiment was performed using a real-time full-ring-array photoacoustic computed tomography (PACT) imaging system, which achieved an imaging depth of 11 mm and a 100 ?m radial resolution.

Because of the fast imaging speed of the full-ring-array PACT system, no animal motion artifact was induced. The frame rate of the system was limited by the laser repetition rate (50 Hz). In addition to anatomical imaging of the blood vessels in the brain, we continuously monitored correlations between the two brain hemispheres in one of the coronal planes. The resting states in the coronal plane were measured before and after stroke ligation surgery at a neck artery.

10064-6, Session 1

Listening to membrane potential: photoacoustic voltage sensitive dye recording

Haichong K. Zhang, Jeeun Kang, Johns Hopkins Univ. (United States); Ping Yan, Univ. of Connecticut School of Medicine (United States); Diane Abou, Johns Hopkins Univ. (United States); Hanh N. D. Le, Univ. of Connecticut School of Medicine (United States); Daniel Thorek, Jin U. Kang, Johns Hopkins Univ. (United States); Albert Gjedde, Univ. of Copenhagen (Denmark); Arman Rahmim, Dean F.

Wong, Johns Hopkins Univ. (United States); Leslie M. Loew, Univ. of Connecticut School of Medicine (United States); Emad M. Boctor, Johns Hopkins Univ. (United States)

Monitoring of the membrane potential is possible using voltage sensitive dyes (VSD), where fluorescence intensity changes in response to neuronal electrical activity. Fluorescence imaging is limited by depth of penetration and sensitivity concerns. Photoacoustic imaging is an emerging modality that enables deep tissue, noninvasive imaging by combining near infrared light excitation and ultrasound detection. In this BRAIN initiative effort, we developed a novel photoacoustic VSD capable of detecting the active potential variation. In the polarized state, this cyanine-based probe enhances photoacoustic intensity while decreasing fluorescence output in a lipid vesicle membrane model. At 9uM concentrated VSD sample, we report a photoacoustic signal improvement of 12.6% in photoacoustic strength, and observe a signal reduction of 40% in fluorescence emission and 3.0% in absorbance. In a theoretical model, the photoacoustic signal increase was estimated to be 12.2%, which was close to the experimental value. These results not only demonstrate the voltage sensing capability of the dye, but also indicate the necessity of considering both fluorescence and absorption energy transfer in order to optimize the characteristics of novel photoacoustic probes. Together, our results demonstrate a promising new class of high sensitivity photoacoustic dyes which enable deep tissue visualization of electrophysiological events.

10064-7, Session 2

Photoacoustic analysis of thyroid cancer in vivo: A pilot study

Jeesu Kim, Pohang Univ. of Science and Technology (Korea, Republic of); Min-Hee Kim, Kwanhoon Jo, Jeonghoon Ha, Dong-Jun Lim, The Catholic Univ. of Korea (Korea, Republic of); Yongmin Kim, Chulhong Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

Thyroid cancer is one of the most prevalent cancers. About 3-8 % of the people in the United States have thyroid nodules, and 5-15 % of these nodules are malignant. Fine-needle aspiration biopsy (FNAB) is a standard procedure to diagnose malignity of nodules. However, about 10-20 % of FNABs produce indeterminable results, which leads to repeat biopsies and unnecessary surgical operations. We have explored photoacoustic (PA) imaging as a new method to identify cancerous nodules. In a pilot study to test its feasibility, we recruited patients with thyroid nodules (currently 37 cases with 22 malignant and 15 benign nodules), acquired in vivo PA and ultrasound (US) images of the nodules in real time using a recently-developed clinical PA/US imaging system, and analyzed the acquired data offline. The preliminary results show that malignant and benign nodules could be differentiated by utilizing their PA amplitudes at different excitation wavelengths. This is the first in vivo PA analysis of thyroid nodules. Although a larger-scale study is needed for statistical significance, the preliminary results show the good potential of PA imaging as a non-invasive tool for triaging thyroid cancer.

10064-8, Session 2

Photoacoustic evaluation of human inflammatory arthritis in human joints

Janggun Jo, Guan Xu, April Marquardt, Gandikota Girish, Xueding Wang, Univ. of Michigan (United States)

Photoacoustic (PA) imaging combined with ultrasonography (US) holds promise to offer a novel and powerful tool for clinical management of inflammatory arthritis, including early detection and treatment monitoring. In this study on human subjects, imaging of peripheral joints with inflammation as confirmed by clinical evaluation and Doppler US was conducted. The blood volume and the blood oxygenation in the segmented synovium were quantified. The results from the student t-test indicate that either of the two functional measures (blood volume and blood oxygenation) can differentiate the arthritic joints from the normal controls, demonstrating that PI imaging integrated with US is capable of identifying and characterizing inflammation in joints based on the detection of hemodynamic biomarkers.

10064-9, Session 2

Optoacoustic mapping of cerebral blood oxygenation in humans

Yuriy Y. Petrov, Donald S. Prough, Irene Y. Petrov, Joan Richardson, Rafael A. Fonseca, The Univ. of Texas Medical Branch (United States); Claudia S. Robertson, Baylor College of Medicine (United States); Rinat O. Esenaliev, The Univ. of Texas Medical Branch (United States)

Noninvasive mapping, monitoring, and imaging of cerebral blood is important for management of patients with traumatic brain injury, stroke, and other neurological conditions. We proposed to use optoacoustic technology for noninvasive, transcranial monitoring, imaging, and mapping. In this work, we developed optoacoustic systems for mapping of cerebral blood oxygenation in humans and tested them in adults and neonates. The systems provide noninvasive, transcranial optoacoustic measurements in the transmission (forward) and reflection (backward) modes in the near infrared spectral range (680-950 nm). Novel, ultra-sensitive optoacoustic probes were built for detection of optoacoustic signals and measurement of blood oxygenation. The signals were detected from large cerebral blood vessels and cerebral tissues. Measurement of the optoacoustic signals at different locations allowed for mapping of cerebral blood oxygenation beneath the skull.

10064-10, Session 2

Quantitative photoacoustic elastography in humans

Pengfei Hai, Yong Zhou, Lei Gong, Lihong V. Wang, Washington Univ. in St. Louis (United States)

The elasticity of biological tissues is directly related to their structures and functions, and altered elastic properties are often associated with pathological states. Inspired by manual palpation, elastography can noninvasively map the elasticity of biological tissue and detect associated diseases. In this work, we report quantitative photoacoustic elastography (QPAE), capable of measuring Young's modulus of human tissue in vivo. By combining photoacoustic elastography with a stress sensor having known stress-strain behavior, QPAE can simultaneously measure strain and stress, from which Young's modulus is calculated. We first applied QPAE to quantify the Young's modulus of tissue-mimicking agar phantoms with different concentrations. The measured values fitted well with both the empirical expectations based on the agar concentrations and those measured in independent standard compression tests. We then demonstrated the feasibility of QPAE by measuring the Young's modulus of human skeletal muscle in vivo. The data showed a linear relationship between muscle stiffness and loading, which agreed well with the literature. The results proved that QPAE can noninvasively quantify the absolute elasticity of biological tissue, thus enabling longitudinal imaging of tissue elasticity. QPAE can be exploited for both preclinical biomechanics studies and clinical applications.

10064-11, Session 2

First patient results of photoacoustic computed tomography imaging of inflamed finger joints in rheumatoid arthritis

Srirang Manohar, Univ. Twente (Netherlands); Linda M Zwiers, Univ. of Twente (Netherlands); Sreedevi Gutta, Indian Institute of Science (India); Peter van Es, Redmar C. Vlieg, Univ. Twente (Netherlands); Hein B. J. Bernelot Moens, Ziekenhuisgroep Twente (Netherlands); Wiendelt Steenbergen, Univ. Twente (Netherlands); Phaneendra K. Yalavarthy, Indian Institute of Science (India); Christoph Brune, Univ. Twente (Netherlands)

Rheumatoid arthritis is a chronic autoimmune disease affecting synovial joints. Early and accurate diagnosis with appropriate therapy is crucial to prevent progressive cartilage damage and onset of irreversible bone damage. It is hypothesized that an early marker for disease activity and severity is the angiogenesis at the synovial membrane associated with inflammation.

We study the feasibility of using photoacoustics in a tomography geometry to image vascular structures in inflamed and healthy finger joints. A laboratory system is used based on a curved ultrasound detector array with 64 elements at center frequency 7.5 MHz (80% bandwidth). Illumination is realized with a pulsed Nd:YAG laser pumping an OPO. The study protocol was approved by the Institutional Medical Ethics Committee. Using excitation at 800 nm we imaged 3 index finger joints of 3 healthy volunteers and 3 index finger joints of patients with inflamed fingers.

We will shortly discuss the challenges in artifact identification and removal, in the imaging of finger joints, and then present the results. We show that there are marked differences between the vascular distributions of healthy and inflamed finger joints. While we could not unambiguously identify the synovial membrane in the patients, we observe indications of restless regions around the inflamed joint where the thickness and tortuosity of the vasculature is increased. We consider these first results to be promising and worthy of further investigation. Future work will concentrate on including more patients and defining images features that may be indicators of the disease.

10064-12, Session 3

In vivo photoacoustic mouse eye imaging of healing after chemical injury and automated eyeball surface estimation based on a random sample consensus algorithm

Seungwan Jeon, Pohang Univ. of Science and Technology (Korea, Republic of); Hyunbeom Song, Seoul National Univ. Hospital (Korea, Republic of); Jaewoo Kim, Pohang Univ. of Science and Technology (Korea, Republic of); Byungjoo Lee, Jeong Hun Kim, Seoul National Univ. Hospital (Korea, Republic of); Chulhong Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

Ocular chemical injury can induce limbal vessel ischemia and neovascularization, but the pathophysiology of the diseases has not been fully understood. To chronically monitor the vascular changes after an alkali burn, we used label-free in vivo optical resolution photoacoustic microscopy (OR-PAM) to image the anterior segment and choroidal vessel. We observed iris blood vessels and choroidal blood vessels clearly through the sclera, even though the choroidal blood vessels were difficult to image by conventional photography. After the alkali burn, we observed neovascularization and limbal vessel ischemia, and successfully traced the vascular structure change during the healing process for 14 days. In addition, we segmented the abnormal new vessels induced by corneal neovascularization and visualized the distances from each PA signal to the eyeball center by using the RANdom SAmple Consensus (RANSAC) method. This iterative parameter estimation algorithm disclosed the mouse eyeball surface. We believe that photoacoustic imaging has valuable potential for revealing the pathophysiology of limbal ischemia and neovascularization.

10064-13, Session 3

Photoacoustic image-guided navigation system for surgery

Sara Park, Pohang Univ. of Science and Technology (Korea, Republic of); Jongseong Jang, Institute of Innovative Surgical Technology, Hanyang Univ. (Korea, Republic of); Jeesu Kim, Pohang Univ. of Science and Technology (Korea, Republic of); Young Soo Kim, Institute of Innovative Surgical Technology, Hanyang Univ. (Korea, Republic of); Chulhong Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

Identifying and delineating invisible anatomical and pathological details during surgery guides surgical procedures in real time. Various intraoperative imaging modalities have been increasingly employed to minimize such surgical risks as anatomical changes, damage to normal tissues, and human error. However, current methods provide only structural information, which cannot identify critical structures such as blood vessels. The logical next step is an intraoperative imaging modality that can provide functional information. Here, we have successfully developed a photoacoustic (PA) image-guided navigation system for surgery by integrating a position tracking system and a real-time clinical photoacoustic/ultrasound (PA/US) imaging system. PA/US images were acquired in real time and overlaid on pre-acquired cross-sectional magnetic resonance (MR) images. In the overlaid images, PA images represent the optical absorption characteristics of the surgical field, while US and MR images represent the morphological structure of surrounding tissues. To test the feasibility of the system, we prepared a tissue mimicking phantom which contained two samples, methylene blue as a contrast agent and water as a control. We acquired real-time overlaid PA/US/MR images of the phantom, which were well-matched with the optical and morphological properties of the samples. The developed system is the first approach to a novel intraoperative imaging technology based on PA imaging, and we believe that the system can be utilized in various surgical environments in the near future, improving the efficacy of surgical guidance.

10064-14, Session 3

Correlations in photoacoustic estimates of tumor oxygenation during novel cancer therapies with power Doppler measurements

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Photoacoustic (PA) imaging of tumor oxygenation can be used to monitor vascular-targeted novel therapies. This study examines how a combination treatment, ultrasound-microbubbles (USMB)/radiation-therapy (XRT) alters oxygen saturation (sO2) estimates, which are then compared to power Doppler (PD) assessments of tumor vascularity.

SCID mice were inoculated with subcutaneous, hind-leg PC3 tumors. The treatment consisted of XRT/MB (XRT: 8Gy/single-fraction; USMB: 3%/500 kHz/570kPa; n=3), USMB (n=3) and XRT (n=5) alone and untreated control (n=5). PA/PD imaging was acquired pre-treatment and 2h/24h post-treatment using the VevoLAZR (21 MHz, 750/850 nm). The volumetric tumor sO2 was quantified using histogram distributions and the average mode was computed. The vascularization index (VI), a PD metric of tumor vessel density, was studied along with the sO2 mode by comparing changes at 2h with pre-treatment.

Mice whose pre-treatment sO2 levels were over 65%, exhibited a 15% drop in oxygenation at 2h, remaining unchanged by 24h. Examining the sO2 and VI relationships revealed differences between the groups. All groups (except control) exhibited a positive correlation when the ΔVI was plotted as a function of ΔsO2 ($r^2 \geq 0.85$). Mice in the XRT/MB group had the largest slope (11.7) suggesting that a change in sO2 was accompanied by the largest change in vessel density. The slope of the USMB and XRT treatments was 5.6 and 2.9, respectively. The combination treatment induced the largest changes in vessel density and sO2. Early PA estimates of tumor oxygenation appear to correlate with the treatment-induced vascular changes. Such measure could potentially be used for predicting treatment outcome.

10064-15, Session 3

Identification and removal of reflection artifacts in minimally-invasive photoacoustic imaging for accurate visualization of brachytherapy seeds

Mithun Kuniyil Ajith Singh, Vinay Parameshwarappa, Univ. Twente (Netherlands); Ellen Hendriksen, Medisch Spectrum Twente (Netherlands); Wiendelt Steenbergen, Srirang Manohar, Univ. Twente (Netherlands)

In brachytherapy, multiple metallic radioactive sources are implanted inside the prostate in and around the tumor region for localized radiotherapy. It is important to accurately visualize seed during the implantation procedure to ensure matching between planned and delivered radiation dose which can be affected by seed migration, prostate motion etc.

Photoacoustic (PA) imaging using an external ultrasound detector with interstitial light delivery via a cutting percutaneous needle is generating much interest in imaging brachytherapy seeds. A challenge is the presence of reflection artifacts caused by the high PA signal from the optical fiber/needle tip reflecting off the seed. These artifacts can appear in the region of interest and confound image interpretation since the appearances of the artifacts is similar to those of seed.

In this work, we apply a new method called PAFUSion (Photoacoustic-guided focused ultrasound) to identify and reduce these reflection artifacts. We present the system comprising of a commercial handheld US imager and linear array, with illumination provided via the cutting needle from a ns pulsed laser. Non-radioactive brachytherapy seeds are implanted in a tissue mimicking phantom and ex vivo porcine tissue. The PAFUSion- corrected imaging results successfully demonstrate that our approach can identify and strongly reduce reflection artifacts in the PA needle. The phantom result also shows that multi-spectral PA can be a useful tool to separate signal from the brachytherapy seeds and other optical absorbers in tissue. By reducing the problem from artifacts, our approach brings interstitial illumination PA imaging into the realm of practical use.

10064-16, Session 3

Non-contact monitoring during laser surgery by measuring the incision depth with air-coupled transducers

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Laser surgery offers important benefits over traditional scalpel-based procedures, which are often associated with excessive mechanical traumatization and bacterial contamination. In laser surgery, the incision is generated by ablating with an intense focused laser beam, which results in vaporization and ejection of tissues. In this way, minimally invasive intervention and less collateral damage than in standard surgeries is achieved, with more efficient hemo- and bacterio-stasis and less postoperative pain and swelling. However, controlling the incision depth is hampered by lack of haptic feedback and real-time monitoring during laser interventions, consequently leading to a high risk of undesired tissue damage. Here, we present a new feed-back sensing method that accomplishes non-contact real-time monitoring of laser ablation procedures. Ablation was performed per-pulse laser energies above 40 mJ. The shock waves emanating from the ablation spot were subsequently detected with air-coupled transducer. The incision depth was then estimated based on the difference in the time-of-flight of the detected shock waves. Experiments in soft and hard tissue samples attained high reproducibility for the real-time depth estimation of the laser-induced cuts. The advantages derived from the non-contact nature of the suggested monitoring approach hold promise for improving the monitoring of laser based surgeries.

10064-17, Session 3

Photoacoustic characterization of the left atrium wall: healthy and ablated tissue

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Radio-frequency ablation (RFA) creates a thermal lesion in the atrial wall, with clearly recognizable optical and structural changes to the tissue. This can be detected by photoacoustic (PA) imaging, and used for monitoring of lesion depth, lesion functionality, and limiting excessive ablation. Porcine left atrium tissue can be split into three visually distinguishable regions, a thick white endocardium, pinkish myocardium and a thin gelatinous epicardium. In this study, we characterize the layered left atrium tissue in terms of the relevant photoacoustic parameters (wavelength, frequency content, imaging depth, lesion contrast). Previous studies in the literature targeted the photoacoustic characterization of fresh and ablated ventricular myocardium in the range of 650nm to 900nm. In this study we target the characterization of fresh and ablated left atrial tissue from 410nm to 1000nm, including the endocardium and epicardium. We generate the

photoacoustic signals using a tunable pulsed laser source, and record those signals using either a broadband 1 mm hydrophone or a L12-3v transducer connected to the Verasonics machine for more realistic conditions. Initial experiments on fresh porcine tissue show that the presence of the endocardium and epicardium layers do affect the photoacoustic signal received. The signal recorded is representative of the difference in optical and mechanical properties between the layers. Ablated and non-ablated tissue also present differences in spectra. The determined optical contrast could be used in the PA monitoring of RFA lesion to monitor the extension of the lesion to the edge of the myocardium-epicardium border avoiding complications related to over ablation.

10064-18, Session 3

Optimizing light delivery for a photoacoustic surgical system

Blackberrie Eddins, Muyinatu A. Lediju Bell, Johns Hopkins Univ. (United States)

Previous work demonstrated the feasibility of photoacoustic imaging to improve pituitary tumor resection by providing real-time, intraoperative visualization of the internal carotid arteries hidden by sphenoid bone. This work explores light delivery optimization to the surgical site. Monte Carlo simulations were employed to study 3D light propagation in tissue, consisting of one or two 4-mm diameter arteries located 3 mm below bone, an absorbing metallic drill contacting the bone surface, and a single light source placed next to the 2.4 mm diameter drill shaft, set 4.95 mm back from the 2.9 mm diameter spherical drill tip. The optimal fiber distance from the drill shaft was 2 mm, determined from the maximum normalized fluence seen by the artery. A single fiber was insufficient to deliver light to arteries separated by a minimum of 8 mm. Using the same drill geometry and the optimal 2 mm fiber-to-drill shaft distance, a 950 nm wavelength Gaussian beam was propagated through one or more 600 micron core diameter optical fibers and detected on the representative bone surface with Zemax ray tracing simulations. When the number of equally spaced fibers surrounding the drill increased from 1 to 10, a single merged optical profile formed with 7 or more fibers, determined by thresholding the resulting light profile images at 1/e times the maximum intensity. The corresponding spot size was 3.9 times larger than that of a single fiber transmitting the same input energy, thus reducing the fluence delivered to the sphenoid bone and enabling higher energies within safety limits. Results are generalizable to multiple interventional photoacoustic applications.

10064-19, Session 3

Ultrasound and photoacoustic imaging to monitor ocular stem cell delivery and tissue regeneration

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Glaucoma is associated with dysfunction of the trabecular meshwork (TM), a fluid drainage tissue in the anterior eye. A promising treatment involves delivery of stem cells to the TM to restore tissue function. Currently histology is the gold standard for tracking stem cell delivery and differentiation. To expedite clinical translation, non-invasive longitudinal monitoring in vivo is desired. Our current research explores a technique combining ultrasound (US) and photoacoustic (PA) imaging to track mesenchymal stem cells (MSCs) after intraocular injection. Adipose-derived MSCs were incubated with gold nanospheres to label cells (AuNS-MSCs) for PA imaging. Successful labeling was first verified with in vitro phantom studies. Next, MSC delivery was imaged ex vivo in porcine eyes, while intraocular pressure was hydrostatically clamped to maintain a physiological flow rate through the TM. US/PA imaging was performed before, during, and after AuNS-MSC delivery. Additionally, spectroscopic PA imaging was implemented to isolate PA signals from AuNS-MSCs. In vitro cell imaging showed AuNS-MSCs produce strong PA signals, suggesting that MSCs can be tracked using PA imaging. While the cornea, sclera, iris, and TM region can be visualized with US imaging, pigmented tissues also produce PA signals. Both modalities provide valuable anatomical landmarks for MSC localization. During delivery, PA imaging can visualize AuNS-MSC motion and location, creating a unique opportunity to guide ocular cell delivery. Lastly, distinct spectral signatures of AuNS-MSCs allow unmixing, with potential for quantitative PA imaging. In conclusion, results show proof-of-concept for monitoring MSC ocular delivery, raising opportunities for in vivo image-guided cell delivery.

10064-20, Session 4

A photoacoustic tool for therapeutic drug monitoring of heparin

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Heparin is used broadly in cardiac, pulmonary, surgical, and vascular medicine to treat thrombotic disorders with over 500 million doses per year globally. Despite this widespread use, it has a narrow therapeutic window and is one of the top three medication errors. The active partial thromboplastin time (aPTT) monitors heparin, but this blood test suffers from long turnaround times, a variable reference range, and limited utility with low molecular weight heparin. Here, we describe an imaging technique that can monitor heparin concentration and activity in real time using photoacoustic spectroscopy via methylene blue as a simple and Federal Drug Agency-approved contrast agent. We found a strong correlation between heparin concentration and photoacoustic signal measured in phosphate buffered saline (PBS) and blood ($R2>0.90$). Clinically relevant concentrations were detected in blood with a heparin detection limit of 0.28 U/mL and a low molecular weight heparin (enoxaparin) detection limit of 72 μg/mL. We validated this imaging approach by correlation to the aPTT (Pearson's $r = 0.86$; $p<0.05$) as well as with protamine sulfate treatment. We then used these findings to create a nanoparticle-based hybrid material that can immobilize methylene blue for potential applications as a wearable/implantable heparin sensor to maintain drug levels in the therapeutic window. To the best of our knowledge, this is the first report to use imaging data to monitor anticoagulation.

10064-21, Session 4

Photoacoustic computed tomography of small-animal wholebody dynamics

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Small animal wholebody imaging, providing physiological, pathological, and phenotypical insights into biological processes, is indispensable in preclinical research. With high spatiotemporal resolution and functional contrast, small animal imaging can visualize biological dynamics in vivo at wholebody scale, which can advance both fundamental biology and translational medicine. However, current non-optical imaging techniques lack either spatiotemporal resolution or functional contrasts, whereas pure optical imaging suffers from either shallow penetration (up to ~1 mm) or a poor resolution-to-depth ratio (~1/3). Here, we present a standalone system that overcomes all the above limitations. Our technology, with unprecedented performance, is envisioned

to complement existing modalities for imaging entire small animals.

As an optical imaging modality, our PACT captures the high molecular contrast of endogenous substances such as hemoglobin, melanin, and lipid, as well as exogenous biomarkers at the whole animal scale with full-view fidelity. Unlike other optical imaging methods, our PACT sees through ~3 cm of tissue in vivo, and acquires cross-sectional images with an in-plane resolution of ~100 μm. Such capabilities allow us to image, for the first time, mouse wholebody dynamics in real time with clear sub-organ anatomical and functional details and without motion artifacts. Our PACT can capture transients of wholebody oxygen saturation, and pulse wave propagation in vivo without labeling. In sum, we expect widespread applications of our PACT as a wholebody imaging tool for small animals in fundamental biology, pharmacology, pathology, oncology, and more.

10064-22, Session 4

Quantitative imaging of tumour vasculature using multispectral optoacoustic tomography (MSOT)

Michal R. Tomaszewski, James Joseph, Univ. of Cambridge (United Kingdom) and Cancer Research UK Cambridge Institute (United Kingdom); Isabel Quirós-Gonzalez, Cancer Research UK Cambridge Institute (United Kingdom); Sarah E. Bohndiek, Univ. of Cambridge (United Kingdom) and Cancer Research UK Cambridge Institute (United Kingdom)

The ability to evaluate tumor oxygenation in the clinic could indicate prognosis and enable treatment monitoring, since oxygen deficient cancer cells are often more resistant to chemotherapy and radiotherapy. MultiSpectral Optoacoustic Tomography (MSOT) is a hybrid technique combining the high contrast of optical imaging with spatial resolution and penetration depth similar to ultrasound. We hypothesized that MSOT could reveal both tumor vascular density and function based on modulation of blood oxygenation.

We performed MSOT on nude mice (n=25) bearing subcutaneous xenograft PC3 tumors using an inVision 256 (iThera Medical). The mice were maintained under inhalation anesthesia during imaging and respired oxygen content was modified from 21% to 100% and back. After imaging Hoechst 33348 was injected to indicate vascular perfusion and permeability. Tumors were then extracted for histopathological analysis and fluorescence microscopy.

Baseline SO2 values at 21% and 100% oxygen breathing showed no relationship with ex vivo measures of vascular density or function. Tumor voxels responding to oxygen challenge were spatially heterogeneous; the fraction responding correlated with Hoechst intensity ($r=-0.53$, $p=<0.0001$). The time to half-maximum of the SO2 change in response to the oxygen gas (T1/2) also correlated with Hoechst intensity ($r=-0.48$, $p=0.01$). The tumors showed significantly slower response kinetics compared to healthy tissue ($T1/2 = 300\pm23$ s vs 74 ± 3 s, $p<0.0001$).

Our results indicate that in subcutaneous prostate tumors, magnitude of the response to oxygen challenge provides insight into tumor vascular function. Future work will include validation using in vivo imaging and protocol optimization for clinical application.

10064-23, Session 4

Photoacoustic pH imaging with SNARF-PAA NP for in vivo tumor

Janggun Jo, Chang Heon Lee, Raoul Kopelman, Xueding Wang, Univ. of Michigan (United States)

Photoacoustic (PA) imaging of pH levels in tumors in vivo was achieved by using polyacrylamide nanoparticles loaded with pH sensitive dye (SNARF). PA images of tumors were acquired at four wavelengths, 565 nm, 576 nm, 584 nm, and 600 nm, which allows removing of the background signal from the blood. The dynamic delivery of the nanoparticles to the tumors, and the pH levels in the tumors at different time points after injection were mapped. The results from the tumors were compared to those from the muscles as controls. The acidic microenvironment in the tumors were confirmed, and validated by the measurements from locally applied pH electrode.

10064-24, Session 4

Simultaneous measurements of total hemoglobin concentration and blood oxygenation with laser diode-based optoacoustic system

Irene Y. Petrov, Donald S. Prough, Yuriy Y. Petrov, Nan Henkel, Roger Seeton, Rinat O. Esenaliev, The Univ. of Texas Medical Branch (United States)

Noninvasive techniques for total hemoglobin concentration (THb) and blood oxygenation (SO2) monitoring often fail to provide accurate measurements. We built a compact, multi-wavelength, nanosecond, fiber-coupled laser diode-based optoacoustic system for noninvasive, accurate monitoring of blood THb and SO2 in blood vessels such as the radial artery. We tested the system in human subjects with different THb. Moreover, we compared performance of the system with that of commercially available systems for measurements of these parameters. The optoacoustic system provided rapid, simultaneous measurement of THb and SO2 with high accuracy. At conditions simulating circulatory shock the optoacoustic system provided more stable monitoring.

10064-25, Session 4

Cerebral blood oxygenation measurements in neonates with optoacoustic technique

Stephen Herrmann, Irene Y. Petrov, Yuriy Petrov, Joan Richardson, Rafael A. Fonseca, Donald S. Prough, Rinat O. Esenaliev, The Univ. of Texas Medical Branch (United States)

Cerebral hypoxia in neonates results in death and severe complications such as cerebral palsy. Currently, no technology is capable of accurate monitoring of neonatal cerebral oxygenation. We proposed to use optoacoustics for this application by probing the superior sagittal sinus (SSS), a large central cerebral vein. We developed and built a multi-wavelength, OPO- and laser diode-based optoacoustic systems for measurement of SSS blood oxygenation in the reflection mode through open anterior and posterior fontanelles and in the transmission mode through the skull in the occipital area. First, the systems were tested in phantoms simulating neonatal SSS. Then, after optimization, we tested them in neonates in NICU. The systems were capable of detecting SSS signals through the open anterior and posterior fontanelles as well as through the skull with high signal-to-noise ratio. Using the signals measured at different wavelengths, the systems provided real-time, continuous oxygenation monitoring with high precision at these locations.

10064-97, Session PSun

X-ray induced acoustic dosimetry using a medical linear accelerator and a focused ultrasound transducer

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Real-time dose monitoring during radiation therapy is extremely important to deliver maximum dose to cancerous tumor site and reduce unintended exposure to surrounding normal tissues. We have successfully developed a therapeutic X-ray induced acoustic (XA) dosimetry system as a promising candidate for a real-time noninvasive dose monitoring during X-ray treatment. The principle of the XA is based on acoustic wave generation from thermoelastic expansion caused by X-ray absorption. The developed XA dosimetry system consists of a medical linear accelerator and a focused ultrasound (US) transducer. An X-ray absorbing target, lead bar, was utilized to verify XA signal acquisition capability of the system. We have successfully synchronized the X-ray accelerator and the US transducer by using a trigger signal generated at each X-ray pulse. We measured the XA signals with varying distances between the lead sample and the US transducer. The travel distances of the XA signals well matched with the actual distances which are measured by pulse-echo signals of US waves. In addition, we verified the XA signal responses to X-ray dose and energy. Amplitude of the XA signal is proportional to both X-ray dose and energy, thus the system can be used as an X-ray dosimetry tool. We believe that the developed XA dosimetry system can be utilized in various clinical and preclinical studies by providing noninvasive real-time measurements of X-ray dose distribution.

10064-98, Session PSun

In vivo photoacoustic imaging of uterine cervical lesion and its image processing based on light propagation in biological medium

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For diagnosis of cervical cancer, screening by use of colposcope and successive biopsy are usually carried out. Colposcope, which is a simple mesoscope, is used to examine surface of the cervix and to find precancerous lesion grossly. However, the diagnosis with colposcopy depends on the skills of the examiner and is inconsistent as a result. Additionally, colposcope lacks depth information. It is known that microvessel density and blood flow in cervical lesion increases associated with angiogenesis. Therefore, photoacoustic imaging (PAI) to detect angiogenesis in cervical lesion has been studied. PAI can diagnose cervical lesion sensitively and provide depth information. Peng and colleagues demonstrated that cervical lesion with CIN2 at a depth of 5 mm was distinguished by PAI ex vivo.

In this study, the authors have been attempting to diagnose the cervical lesion and cancer quantitatively. By use of the PAI and ultrasonography system with transvaginal probe developed by Fujifilm Corporation, more than PA images of 50 cases with the reports of cytohistology and histopathology have been acquired. The results of the cervical lesion and cancer diagnoses with PAI will be reported in the presentation.

For quantitative photoacoustic imaging, it is required to take the light propagation in biological medium into account. The processing of the PA image of cervix by use of the simulation of light propagation based on finite element method has been also tried in this study. Numerical simulation, phantom experiment and in vivo imaging with the image processing will be also reported.

10064-99, Session PSun

Photoacoustic imaging of intracardiac medical devices using internal illumination of carbon nanotube: PDMS composite coatings

Wenfeng Xia, Sacha Noimark, Efthymios Maneas, Ivan P. Parkin, Sebastien Ourselin, Univ. College London (United Kingdom); Malcolm Finlay, Queen Mary, Univ. of London (United Kingdom); Adrien E. Desjardins, Univ. College London (United Kingdom)

Accurate localisation of medical devices such as needles and catheters is of crucial importance for a wide range of ultrasound-guided interventions. As these devices are introduced into the body, they can readily stray from the ultrasound imaging plane and visibility can be lost at steep angles when ultrasound is reflected outside the aperture of the imaging probe. Uncertainty about the location of the medical device tip can lead to complications and procedural inefficiencies. In this study, we investigated visualisation of medical devices by photoacoustic excitation of optically absorbing coatings. Photoacoustic excitation light was provided through optical fibres positioned within a cardiac needle and a steerable-tip catheter. These fibres illuminated elastomeric composite coatings within the devices, which comprised functionalised carbon nanotubes and polydimethylsiloxane (PDMS). Using a swine heart model, photoacoustic images were received with a clinical ultrasound system in conjunction with a transoesophageal imaging probe, and co-registered B-mode ultrasound images were acquired. In the photoacoustic images, prominent signals were obtained from the composite coatings within the medical devices. The spatial locations from which the signals were derived corresponded well to the B-mode ultrasound images. The photoacoustic and ultrasound images were complementary: the former allowed for unambiguous identification of the medical device tips, and the latter provided anatomical information. This study demonstrated that photoacoustic imaging could play a useful role with medical device imaging during minimally invasive cardiac procedures, and that it can be performed with clinical ultrasound scanners for compatibility with current workflow.

10064-100, Session PSun

Possibility of transrectal photoacoustic imaging-guided biopsy for detection of prostate cancer

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Transrectral ultrasonography (TRUS) -guided prostate biopsy is mandatory for histological diagnosis in patients with elevated serum PSA (prostate-specific antigen), but its diagnostic accuracy is not satisfactory, therefore, considerable number of patients is forced to have unnecessary repeated biopsy.

Photoacoustic (PA) imaging has a feature to visualize distribution of hemoglobin clearly. So there is a potential to acquire different map of small vessel network between cancerous and normal tissue. We developed an original TRUS-type PA probe consisting of micro-convex array transducer with optical illumination system provided co-registered PA and ultrasound images. The purpose of this study is to demonstrate clinical feasibility of the transrectral PA image. Obtained prostate biopsy cores were stained with anti-CD34 antibodies as a marker of endothelium of the blood vessel in order to find a pattern of map of small vessel network, which allows for imaging-based identification of prostate cancer. We demonstrated TRUS-

merged-with-PA imaging guided targeted biopsy combined with standard biopsy for capturing the clinically significant tumors. This study describes initial results with TRUS-merged-with-PA imaging guided targeted biopsy.

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10064-101, Session PSun

Accuracy of vessel separation distance measurements for photoacoustic image guidance with and without a teleoperated da Vinci surgical system

Neeraj Gandhi, Blackberrie Eddins, Sungmin Kim, Peter Kazanzides, Muyinatu A. Lediju Bell, Johns Hopkins Univ. (United States)

Minimally invasive surgery carries the risk of rupturing major blood vessels, such as the internal carotid arteries hidden by bone in endonasal transsphenoidal surgery. Surgeons currently use preoperative CT images and an intraoperative endoscopic video feed to determine vessel locations. However, CT images are less accurate as the surgery progresses and endoscopic images are not always sufficient to visualize hidden vessels. We propose photoacoustic imaging to determine the distance between vessels and thereby locate safe areas for incisions. Our photoacoustic system included an Alpinion ECube 12R ultrasound system, L3-8 transducer (3-8 MHz bandwidth), and a laser (either 905-nm wavelength pulsed laser diode or 1064-nm wavelength Nd:YAG laser) coupled to a 0.5 NA optical fiber. A custom phantom was designed and manufactured for modular placement of blood vessels. Experiments were conducted with two 3.18-mm diameter vessel-mimicking targets separated by 10.06, 14.46, 15.43, and 19.63 mm. Photoacoustic images were acquired as the fiber was swept across the vessels with and without teleoperation of the fiber using a research da Vinci Surgical System. Compounded images from a single sweep show all vessel boundaries in a single image. Known vessel separations were compared to the measured distance between the brightest pixels in regions of interest surrounding each vessel boundary. The resulting RMS error was 0.41 +/- 0.26 mm and 0.61 +/- 0.26 mm without and with robotic control, respectively. Results indicate that photoacoustic imaging can be used to determine blood vessel locations for real-time path planning in multiple robotic and non-robotic interventional photoacoustic applications.

10064-102, Session PSun

Interventional multispectral photoacoustic imaging of the epidural space

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Injections of anaesthetics into the epidural space are widely performed to relieve pain. Identification of the epidural space with a needle is commonly performed with the “loss-of-resistance” technique, which involves an injection of saline or air. Visualisation of the epidural space in real time could significantly improve procedural success, especially with abnormal anatomy. Ultrasound imaging can help, but anatomical constraints such as obesity cause limitations in its’ ability to visualise the epidural space. Here, for the first time, we investigated the use of photoacoustic imaging to identify the epidural space using an interventional multispectral photoacoustic (IMPA) imaging system. It was hypothesised that photoacoustic image contrast for epidural veins and adipose tissue could be obtained with optical absorption of haemoglobin and lipids. Light pulses with wavelengths in the ranges of 750 to 850 nm and 1160 to 1260 nm were delivered through an optical fibre positioned within a spinal needle to illuminate the epidural space in a swine model. Co-registered B-mode ultrasound images were acquired. Spectral unmixing of the multispectral photoacoustic images revealed prominent distributions of lipids and haemoglobin in the epidural space at a depth of 35 mm, with a needle-to-target distance of up to 1 cm, as determined by X-ray fluoroscopy images. We conclude that IMPA could be a useful imaging modality to guide placement of needles into the epidural space, by providing information complementary to conventional B-mode ultrasound imaging, X-ray fluoroscopy, and loss-of-resistance.

10064-103, Session PSun

Deep tissue photoacoustic imaging at 1064 nm using a contrast agent based on phosphorus phthalocyanine formulation

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Although photoacoustic computed tomography (PACT) operates with high spatial resolution in biological tissues deeper than other optical modalities, light scattering is a limiting factor. The use of longer near infrared wavelengths reduces scattering. Recently, the rational design of a stable phosphorus phthalocyanine (P-Pc) with a long wavelength absorption band beyond 1000 nm has been reported. Here, we show that when dissolved in liquid surfactants, P-Pc can give rise to formulations with absorbance of greater than 1000 (calculated for a 1 cm path length) at wave-lengths beyond 1000 nm. Using the broadly accessible Nd:YAG pulse laser emission output of 1064 nm, P-Pc could be imaged through 11.6 cm of chicken breast with PACT. P-Pc accumulated passively in tumors following intravenous injection in mice as observed by PACT. Following oral administration, P-Pc passed through the intestine harmlessly, and PACT could be used to non-invasively observe intestine function. When the contrast agent placed under the arm of a healthy adult human, a PACT transducer on the top of the arm could readily detect P-Pc through the entire 5 cm limb. Thus, the approach of using contrast media with extreme absorption at 1064 nm readily enables high quality optical imaging in vitro and in vivo in humans at exceptional depths.

10064-104, Session PSun

A novel compact linear-array based photoacoustic handheld system towards clinical translation for sentinel lymph node mapping

Li Mucong, Chengbo Liu, Liang Song, Xiaojing Gong, Shenzhen Institutes of Advanced Technology (China)

Breast cancer is the leading threat to women health around the world. Sentinel lymph node (SLN) biopsy has become the standard of care for the staging of this disease over the past few decades. During this procedure, radioactive tracers and dyes are injected and an open surgery is required to identify the dye accumulated SLNs. Photoacoustic imaging, as a rapidly growing technology, is gaining widespread attention due to its excellent imaging contrast as well as high resolution for deep imaging. Previously, several groups have explored the feasibility of developing commercial ultrasound based PA systems for identifying SLNs noninvasively to potentially eliminate the need for radioactive tracers and open surgeries. In this study, we developed a handheld ultrasound linear-array based real-time photoacoustic tomography system. Compared with previously reported studies, this system has several unique features and/or advantages: (1) the imaging probe is very compact and user-friendly; (2) laser illumination and ultrasonic detection are achieved co-axially, enabling high signal-to-noise ratio under relatively low laser energy excitation; (3) GPU-based

reconstruction is developed, enabling a real-time imaging and display at a frame rate of 20 Hz. The system was validated with both in vitro and in vivo studies, by imaging a black tape in intralipid solution as well as rat SLNs in vivo, respectively. An in vivo imaging depth of ~2 – 3 cm was demonstrated by placing a thick chicken breast tissue layer on top of the skin surface of the rats. We believe that the developed handheld linear-array photoacoustic imaging system is of great potential to be further translated for clinical applications.

10064-105, Session PSun

Functional photoacoustic tomography for neonatal brain imaging: developments and challenges

Ali Hariri, Parsa Omidi, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States)

Neurodevelopmental outcome after premature birth is complex, and understanding the neurobiological mechanisms that support brain development after premature birth is crucial. Brain imaging could potentially offer a solution. Existing high-resolution brain imaging modalities such as x-ray CT and MRI are expensive and employ bulky and generally non-portable imaging instrumentation. Moreover, x-ray CT employs ionizing radiation and is therefore unacceptable for structural and functional brain imaging in preterm infants. Ultrasonography provides mostly morphological and limited functional information. The goal of this study is to implement a functional imaging system to monitor infant brains noninvasively using photoacoustic technology. In addition to several developments, the proposed system in here, is developed based on multiple single element ultrasonic transducers that are configured hemispherically. A new side light illumination scheme as well as a semi-dry coupling configuration is used in this system. A novel three dimensional dictionary based compressed sensing reconstruction algorithm is used for image reconstruction.

10064-106, Session PSun

Dry coupling for whole-body small-animal photoacoustic computed tomography

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Whole-body small-animal imaging is widely used in biomedical research for studying and modeling human disease. Recently, there has been increased interest in whole-body small-animal photoacoustic tomography (PAT). PAT breaks the optical diffusion limits and yields high-resolution images in diffusive optical regimes. Over the past few years, multiple whole-body small-animal PAT systems have been implemented using different acoustic coupling media, light delivery systems, and acoustic detection designs. However, all existing whole-body small-animal photoacoustic imaging systems use water as a direct-contact coupling medium, which can induce anxiety and water-immersion wrinkling in mice. Both of these factors can render physiological measurements inaccurate in various ways, such as by decreasing T-cell blastogenesis, altering blood flow velocity, and inducing vasoconstriction.

Here, we report a ring-shaped dry-coupled confocal photoacoustic computed tomography system (RDC-PACT) that overcomes these limitations. The dry acoustic coupler is made of a tubular elastic membrane enclosed by a closed transparent water tank. The tubular membrane ensures water-free contact with the animal, and the closed water tank allows pressurization for animal stabilization. The dry coupler was tested using a whole-body small-animal ring-shaped photoacoustic computed tomography system. Dry coupling was found to provide image quality comparable to that of conventional water coupling.

10064-107, Session PSun

A suite of phantom-based test methods for assessing image quality of photoacoustic tomography systems

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As Photoacoustic Tomography (PAT) matures and undergoes clinical translation, objective performance test methods are needed to facilitate device development, regulatory clearance and clinical quality assurance. For mature medical imaging modalities such as CT, MRI, and ultrasound, tissue-mimicking phantoms are frequently incorporated into consensus standards for performance testing. A well-validated set of phantom-based test methods is needed for evaluating performance characteristics of PAT systems. To this end, we have constructed phantoms using a custom tissue-mimicking material based on PVC plastisol with tunable, biologically-relevant optical and acoustic properties. Each phantom is designed to enable quantitative assessment of one or more image quality characteristics including 3D spatial resolution, spatial measurement accuracy, ultrasound/PAT co-registration, uniformity, penetration depth, geometric distortion, sensitivity, and linearity. Phantoms contained targets including high-intensity point source targets and dye-filled tubes. This suite of phantoms was used to measure the dependence of performance of a custom PAT system (equipped with four interchangeable linear array transducers of varying design) on design parameters (e.g., center frequency, bandwidth, element geometry). Phantoms also allowed comparison of image artifacts, including surface-generated clutter and bandlimited sensing artifacts. Results showed that transducer design parameters create strong variations in performance including a trade-off between resolution and penetration depth, which could be quantified with our method. This study demonstrates the utility of phantom-based image quality testing in device performance assessment, which may guide development of consensus standards for PAT systems.

10064-108, Session PSun

A monomeric water-soluble NIR-absorbing porphyrin derivative as in vivo photoacoustic tomography contrast agent

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The synthesis of a monomeric water-soluble PEGylated (polyethylene glycol) quinoline-annulated porphyrin and its evaluation as a photoacoustic (PA) contrast agent is presented. The PEGylated mono-quinoline-annulated porphyrin was prepared from meso-tetrakis(p-methoxyphenyl)-porphyrin. Similar to the previously prepared meso-phenylquinoline-annulated porphyrins, it possesses intense absorption in the near infrared range (?max > 750 nm), exhibits very little fluorescence but a strong PA response. The dye demonstrated high solubility (100 mg/ml) and stability in water and phosphate-buffered saline (PBS). No toxicity sign was observed in BALB/c mice. The PA generation efficiency of the dye was measured to be approximately 4 times higher than fresh rat blood. The dye provides distinguishable PA signal from inside a tube positioned 2.5 cm below the surface of a scattering medium, as opposed to the noise-level signal from blood at similar absorbance. The enhancement of in vivo photoacoustic tomography (PAT) images of implanted murine tumors was compared between the retro-orbital injection of 100 ?l solutions of the dye (n = 9) and indocyanine green (ICG) (n = 3) at similar absorbance. Injection of the dye provides 3.8 times improvement in the PA signal, while ICG provides a 1.6-fold improvement. Furthermore, the dye demonstrates a slower washout than ICG within 45 minutes following injection. The biodistribution of the contrast agent was studied by injection of a fluorescent-tagged

derivative prepared by conjugation of the contrast agent with a BODIPY fluorophore. Ex vivo fluorescence images of the mouse organs acquired 48 hours after injection of the fluorescent-tagged dye (n = 3) indicate a strong accumulation in the tumor. Moreover, mass spectroscopy analysis of a CH2Cl2 extract of the mouse urine after injection revealed an unaltered renal filtration of the contrast agent.

10064-109, Session PSun

N-doped carbon nanodots for non-invasive photoacoustic imaging and photothermal therapy

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We have synthesized nitrogen doped carbon nanodots (N-CNDs) for photoacoustic (PA) imaging and photothermal therapy (PTT) by controlling nitrogen source and by carbonizing organic acids. In this process, oleylamine served as a surfactant to suppress undesired inter-particle agglomeration during carbonization, and it was subsequently replaced by ethanolamine to endow our N-CNPs with water solubility. The N-CNDs show strong optical absorbance in the near-infrared region with great photostability, and biodegradability. Therefore, the PA signals from N-CNDs were high enough to detect inside living animals, and minimally invasive PTT using N-CNDs was possible. To investigate the performance of N7-CNP as contrast agent, we have directly compared the PA signals with the popular conventional PA contrast agents such as gold nanorods and methylene blue. At the same optical density, the normalized PA signals amplitude of N-CND was approximately two times higher than gold nanorods and methylene blue with the strong stability of the PA response from high energy pulsed laser. Additionally, to verify the biodegradability and potential application of N-CNDs as a PA imaging contrast agent, we performed time-resolved PA imaging of sentinel lymph nodes (SLNs) and assessed renal clearance after hypodermic injection. SLN and vascular networks were photoacoustically visualized by an acoustic-resolution reflection-mode PA imaging system at 680 nm optical wavelength. Furthermore, we conducted whole body PA imaging after N-CND subcutaneous injection for revealing the body distribution and clearance of N-CND. Finally, we further investigated the in vivo photothermal therapy of balb/c nude xenograft HepG2 tumor model mice using N-CND.

10064-110, Session PSun

Dependence of photoacoustic signal generation characteristics on fluorescence quantum yields of small organic molecule based contrast agents

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Photoacoustic (PA) imaging is advantageous in contrast agent imaging because of high spatial resolution at depth more than several millimeter inside biological tissues. To detect small tumors specifically, we are developing small ortanic molecule-based activatable PA probe with mechanism similar to that of the enzyme-activatable fluorescence probe that have successfully used for rapid fluorescence imaging of small tumors (Urano et al, Sci. Transl. Med., 2011). The probe can be imaged also by fluorescence imaging and the fluorescence image can be merged onto the PA images. To extend the imaging depth by increasing PA signal intensity, PA probe that produce PA signals efficiently is required.

To select contrast agents suitable for PA probe, we synthesized small-organic molecule-based contrast agents with various absorption spectra and fluorescence quantum yields ? and then we exhaustively evaluated their PA signal generation characteristics including PA signal generation efficiencies. To analyze PA signal generation efficiencies precisely, the absolute values of PA signal pressures produced from aqueous solutions of the contrast agents were measured by P(VDF-TrFE) piezoelectric film acoustic sensor that was developed and calibrated by us.

As a result, PA signal generation efficiencies of the contrast agents were almost proportional to (1??). Thus, as opposed to fluorescence probes, PA probes should have low fluorescence quantum yields. By considering the result and other characteristics including excitation wavelengths, we could single out the contrast agent to use for PA probe. Currently, we are synthesizing the activatable PA probe and planning a clinical study of small cancer detection.

10064-111, Session PSun

Design of photoacoustic endoscope (PAE) system using MEMS mirror and transducer array

Tong Li, Xiyu Duan, Haijun Li, Gaoming Li, Kenn R. Oldham, Thomas D. Wang, Univ. of Michigan (United States)

Photoacoustic imaging, because of its advantage of combining the high spatial resolution and image depth of ultrasound and the high contrast of light, has a very promising potential of being implemented into an endoscope. Currently Photoacoustic Endoscopes (PAE) using a single element ultrasound transducer and rotating scanning mirror have been developed. However, the unfocused laser in these designs make the resolution limited by the ultrasound transducer, and the rotating scanning mechanisms cannot achieve 2D scanning without a pulling-back stage. By combining a transducer array and a MEMS scanning mirror, 2D laser scanning and 3D acoustic focusing can be achieve simultaneously without complex component design, making it easier to be miniaturized. Previously our group developed a 4.2 mm side-view confocal endomicroscope using a fast and compact MEMS scanning mirror, which is able to acquire real time NIR fluorescence images and produce optical sections of epithelium in vivo. This paper presents the design of a novel PAE system based on our previous design, integrating array transducer and MEMS mirror technology. Modeling of laser scanning and array transducer signal detection was first done to help understand the imaging process. Then simulation was run to determine the key parameters of the MEMS mirror and array transducer. A prototype tabletop system using galvanometer mirror and three different kinds of ultrasound detectors was also built to verify the concept and collect testing data. Photoacoustic images of different phantoms were acquired to test the performance of the system.

10064-112, Session PSun

Towards non-contact photo-acoustic endoscopy using speckle pattern analysis

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Photoacoustic Tomography combines the advantages of optical and acoustic imaging as it makes use of the high optical contrast of tissue and the high penetration depth of ultrasound. Furthermore, high penetration depths in tissue in the order of several centimeters can be

achieved by the combination of these modalities. Extensive research is being done in the field of miniaturization of photoacoustic devices, as photoacoustic imaging could be of significant benefits for the physician during endoscopic interventions. All the existing miniature systems are based on contact transducers for signal detection that are placed at the distal end of an endoscopic device. This makes the manufacturing process difficult and impedance matching to the inspected surface a requirement. The requirement for contact limits the view of the physician during the intervention. Consequently, a fiber based non-contact optical sensing technique would be highly beneficial for the development of miniaturized photoacoustic endoscopic devices. This work demonstrates the feasibility of surface displacement detection using remote speckle-sensing using a high speed camera and an imaging fiber bundle that is used in commercially available video endoscopes. The feasibility of sensing of displacement is demonstrated by analysis of loudspeaker membrane oscillations. Since the usability of the remote speckle-sensing for photo-acoustic signal detection was already demonstrated, the fiber bundle approach demonstrates the potential for non-contact photoacoustic detections during endoscopy.

10064-113, Session PSun

Photoacoustic tomography of intraocular tumors: Investigation on laser safety

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Intraocular tumors are relatively rare, but life-threatening conditions. Patients with uveal melanoma, which accounts for 80% of primary intraocular tumors, have a 5-year survival rate of about 15% percent once the tumors have metastasized. Current clinical diagnostic modalities are unable to assess the molecular components inside the tumors, which is an important factor in differential diagnosis. In our previous study, we have quantitatively differentiated uveal melanoma and retinoblastoma in enucleated human eye globes by their molecular components and mircoarchitectures using photoacoustic imaging. Illumination through cornea was avoided as the lens could focus the light onto retina and cause vision damage. Currently there is no safety limit to optical energy delivered through sclera. This study investigates the safety limit to optical density for photoacoustic imaging of intraocular tumors through sclera with rabbits. An optical guide with diameter of 1/2 inch will deliver the illumination to the surfaces of the eye globes. The rabbit eyes will be exposed to optical illumination at 700-950 nm with a step size of 50 nm and 5 ns pulse width. Illumination at each wavelength will be repeated for 10 times to mimic the averaging during data acquisition. Retinal electroretinogram will be performed to evaluate the retinal toxicity after each scanning. The rabbit eyes will be harvested and examined by histopathology. We will start at energy density of 20mJ/cm2 and reduce the energy if vision damage is observed. We expect to find the safety limit by scanning a total of 20 rabbits, i.e. 40 eyes.

10064-114, Session PSun

Study of data analysis methods in functional connectivity photoacoustic tomography (fcPAT)

Afsoon Khodaee, Ali Hariri, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States)

Resting-state functional connectivity (RSFC) is a method to monitor the health of the brain and find out abnormalities in brain networks. Recently functional connectivity photoacoustic tomography (fcPAT) has been used to study RSFC in the mouse brain. The current method of RSFC data analysis is called “seed-based”. This method is not data-driven, and involves user intervention. Alternative signal processing approaches, such as singular value decomposition (SVD) and independent component analysis (ICA), will be explored to complement and cross validate the seed-based approach, possibly substituting them for the seed-based method. The methods are implemented and applied on the fcPAT data of a mouse brain.

10064-115, Session PSun

A cost-effective functional connectivity photoacoustic tomography (fcPAT) of the mouse brain

Ali Hariri, Parsa Omidi, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States)

The increasing use of mouse models for human brain disease studies, coupled with the fact that existing high-resolution functional imaging modalities cannot be easily applied to mice, presents an emerging need for a new functional imaging modality. Utilizing a novel light illumination scheme and a moving multiple-single-transducer ultrasonic detection, we imaged spontaneous cerebral hemodynamic fluctuations and their associated functional connections in the mouse brain. The images were acquired noninvasively with a large field of view, and a high spatial resolution. Correlations were investigated inter-hemispherically between bilaterally homologous regions, as well as intra-hemispherically within the same functional regions. The functional connectivity in different regions are studied. The resultant map can then be used in the study of brain disorders such as stroke, Alzheimer’s, schizophrenia, multiple sclerosis, autism, and epilepsy. Our experiments show that photoacoustic technology is able to detect connectivities between different functional regions, promising a powerful functional imaging modality for future brain research.

10064-116, Session PSun

Comparative study on similarity metrics for seed-based analysis of functional connectivity photoacoustic tomography images

Afsoon Khodaee, Ali Hariri, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States)

Seed-based correlation analysis is one of the most popular methods to explore the functional connectivity in the brain. Based on the time series of a seed, i.e., small regions of interest, connectivity is computed as the correlation of time series for all other pixels in the brain. Similarity metrics to measure the similarity between time courses of different seeds, plays an important role in the detection of functional connectivity maps. In this study, we investigate the performance of six similarity metrics including Pearson correlation, Kendall, Spearman, Goodman-Kruskal Gamma, normalized cross correlation and coherence analysis to determine their performance for the functional connectivity photoacoustic tomography (fcPAT) signals/images. The methods are implemented and applied on the fcPAT data of a mouse brain. We also add noise to the fcPAT data and explore the noise tolerance of these metrics.

10064-117, Session PSun

Toward high-speed transcranial photoacoustic imaging using compact near-infrared pulsed LED illumination system

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Quantification of brain function is a significant milestone towards

understanding of the underlying workings of the brain. Photoacoustic (PA) imaging is the emerging brain sensing modality by which the molecular light absorptive contrast can be non-invasively quantified from deep-lying tissue (~several cm). In this BRAIN initiative effort, we propose high-speed transcranial PA imaging using a novel, compact pulsed LED illumination system (Prexion Inc., Japan) with 200-uJ pulse energy for 75-ns duration, and pulse repetition frequency (PRF) up to 4kHz at near-infrared (NIR) wavelengths of 690-nm and 850-nm switchable in real-time. To validate the efficacy of the proposed system, preliminary ex vivo experiments were conducted with mice skull and human temporal bone, which included vessel-mimicking tubes filled with 10% Indian Ink solution and light absorptive rubber material, respectively. The results indicated that significant PA contrast, 150% signal-to-noise ratio (SNR), can be achieved through the mice skull only with 64 subsequent frame averaging. The minimal number of frames for averaging required was only 16 to generate signal above background noise, leading to 250 Hz frame rate in the strictest temporal frame separation. Furthermore, distinguishable PA contrast was achieved with human temporal bone with 64-frame averaging. Overall, the preliminary results indicate that the LED illumination system can be a cost-effective solution for high-speed PA brain imaging in preclinical and clinical applications, compared to expansive and bulky Nd:YAG laser systems commonly used in PA imaging.

10064-118, Session PSun

Three-dimensional optoacoustic mesoscopy of the tumor heterogeneity in vivo using high depth-to-resolution multispectral optoacoustic tomography

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Multispectral optoacoustic mesoscopy (MSOM) has been recently introduced for cancer imaging, it has the potential for high resolution imaging of cancer development in vivo, at depths beyond the diffusion limit. Based on spectral features, optoacoustic imaging is capable of visualizing angiogenesis and imaging cancer heterogeneity of malignant tumors through endogenous hemoglobin and exogenous contrast agents such as gold nanoparticles. However, high-resolution structural and functional imaging of whole tumor mass is limited by modest penetration and image quality, due to the insufficient capability of ultrasound detectors and the two-dimensional scan geometry. In this study, we introduce a novel multi-spectral optoacoustic mesoscopy (MSOM) for imaging subcutaneous or orthotopic tumors implanted in lab mice, with the high-frequency ultrasound linear array and a conical scanning geometry. Detailed volumetric images of vasculature, oxygen saturation of tissue, and the bio-distribution of gold nanoparticles in the entire tumors are obtained in vivo, at depths up to 10 mm and an isotropic resolution approaching 70?m. This unprecedented performance enables the visualization of vasculature morphology, hypoxemia conditions and vascular permeability for gold nanoparticles, and has been verified with microscopic studies ex vivo. These findings demonstrate the potential of MSOM for preclinical oncological studies in deep solid tumors to facilitate the characterization of tumor's angiogenesis and the evaluation of treatment strategies.

10064-119, Session PSun

State-of-the-art of photoacoustic imaging: applications and market trends

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Although the PhotoAcoustic effect was observed by Graham Bell in 1880, the first applications (gas analysis) occurred in 1970's using the required energetic light pulses from lasers.

During mid 1990's medical imaging research begun to use PhotoAcoustic effect and in vivo images were obtained in mid-2000's. Since 2009, the number of publications and patents related to PhotoAcoustic Imaging (PAI) has dramatically increased. PAI machines for pre-clinical and small animal imaging have been being used in a routine way for several years.

Based on its very interesting features (non ionizing radiation, non-invasive, high depth resolution ratio, scalability, moderate price) and because it is able to deliver not only anatomical, but functional and molecular information, PAI is a very promising clinical imaging modality. It penetrates deeper into tissue than OCT (Optical Coherence Tomography) and provides a higher resolution than ultrasounds.

Our study analyzes the different approaches such as photoacoustic computed tomography, 3D photoacoustic microscopy, multispectral photoacoustic tomography and endoscopy with the recent and tremendous technological progress over the past decade : advances in image reconstruction algorithms, laser technology, ultrasound detectors and miniaturization. We analyze which medical domains and applications are the most concerned and explain what should be the forthcoming medical system in the near future. We point out the market accessibility (patents, regulations, clinical evaluations, pricing) and what should be, quantitatively and qualitatively, the PAI medical markets and its main trends.

The PAI is one of the most growing imaging modality and some innovative clinical systems are planned to be on the market in 2017.

10064-120, Session PSun

Utilising the radiative transfer equation in quantitative photoacoustic tomography

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Quantitative photoacoustic tomography (QPAT) is an emerging imaging technique aimed at estimating the optical parameters inside tissue from photoacoustic images which are formed by combining optical information and ultrasonic propagation. Thus, the method proceeds from photoacoustic tomography by taking the estimated initial pressure distributions as data and estimating the absolute values of the optical parameters. This optical parameter estimation problem is an ill-posed inverse problem, and thus it is sensitive to measurement and modelling errors. Therefore, light propagation and absorption in QPAT imaging situation needs to be accurately modelled.

A widely accepted model for light propagation in biological tissue is the radiative transfer equation (RTE). However, it is computationally expensive, and therefore its approximation in strongly scattering medium, the diffusion approximation, is often utilised in biomedical imaging. In QPAT, however, the target size is small compared to the average scattering length, and thus the diffusion approximation is not a valid approximation.

In this work, we study numerical solution of the RTE based on finite element method and utilising it in QPAT. Various imaging situations, including one-side illuminations, are investigated.

10064-121, Session PSun

Analysis of negatively focused detectors in optoacoustic imaging

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In optoacoustic tomography, negatively focused detectors may be used for improving the tangential image resolution while preserving a high signal-to-noise ratio (SNR). Commonly, image reconstruction in such scenarios is facilitated by the use of the virtual-detector approach. Although the validity of this approach has been experimentally verified, it is based on an approximation whose effects on the optoacoustic reconstruction have not yet been studied.

In this work, we analyze analytically the response of negatively focused acoustic detectors in both time and frequency domains . Based on this analysis, simple tradeoffs between the detector size, curvature, and sensitivity are formulated. In addition, our analysis reveals the geometrical underpinning of the virtual-detector approximation and quantifies its deviation from the exact solution. The effect of the error involved in the virtual-detector approximation is studied in image reconstruction simulations and its effect on image quality is shown.

Our analysis reveals that negatively focused detectors are, in many cases, superior to flat detectors in terms of image resolution and SNR. While tangential resolution of images obtained with large flat detectors may be improved by the use of deblurring algorithms, this procedure often comes at the expense of SNR. The theoretical tools developed in this work give valuable insight into the mechanisim of negatively focused detection and may be used in the design of new optoacoustic detection geometries as well as for improved image reconstruction.

10064-122, Session PSun

Coherent-weighted three-dimensional image reconstruction in linear-array-based photoacoustic tomography

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While the majority of photoacoustic imaging systems used custom-made transducer arrays, commercially-available linear transducer arrays hold the benefits of affordable price, handheld convenience and wide clinical recognition. They are not widely used in photoacoustic imaging primarily because of the poor elevation resolution. Here, without modifying the imaging geometry and system, we propose addressing this limitation purely through image reconstruction. Our approach is based on the integration of two advanced image reconstruction techniques: focal-line-based three-dimensional image reconstruction and coherent weighting. We first numerically validated our approach through simulation and then experimentally tested it in phantom and in vivo. Both simulation and experimental results proved that the method can significantly improve the elevation resolution (up to 4 times in our experiment) and enhance object contrast.

10064-123, Session PSun

Improvement of resolution in linear-array-based photoacoustic computed tomography using a novel adaptive weighting method

Parsa Omidi, Ali Hariri, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States)

Linear-array-based photoacoustic computed tomography is a popular methodology for deep and high resolution imaging. However, issues such as phase aberration, side-lobe effects, and propagation limitations deteriorate the resolution. The effect of phase aberration due to acoustic attenuation, and constant assumption of the speed of sound (SoS) can be reduced by applying an adaptive weighting method such as the coherence factor (CF). Utilizing an adaptive beamforming algorithm such as minimum variance (MV) can improve the resolution by eliminating the side-lobes. Regarding propagation limitation, invisibility of directional objects emitting parallel to the detection plane, stretched in the direction perpendicular to the detection plane degrades the resolution. In this study, we propose a novel adaptive weighting algorithm in which weighs are assigned to different positions of the linear array, based on the histogram of oriented gradient (HOG). Simulation results obtained from a synthetic phantom and also those obtained from ex-vivo experiments show the superior performance of the proposed method over the existing reconstruction methods.

10064-124, Session PSun

Variational photoacoustic image reconstruction with spatially resolved projection data

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In our work, we show the impact of successful edge-preserving regularization methods such as Total Variation (TV) and Total Generalized Variation (TGV) on photoacoustic image (PAI) reconstruction of spatially resolved projection data. This data was obtained by recording snap-shots of the diverging acoustic field at a defined temporal delay with respect to the photoacoustic excitation using a CCD-camera part of an optical phase contrast ultrasound detection system.

We formulate this model as Kp(t=0)=p(t), where p(t) is the measured acoustic field at time delay t, p(t=0) is the initial acoustic field and K is the forward operator modeling the wave propagation. The goal is to find the initial acoustic field p(t=0). However, this problem cannot be solved directly due to the ill-possessedness. While simple reconstruction methods like least-squares solutions suffer from noise and image artifacts, we impose a-priori knowledge by adding proper regularization R(p(t=0)) such as TV and TGV and solve a convex variational model of following form using the primal-dual algorithm:

minp(t=0) ||Kp(t=0)-p(t)||^2 + R(p(t=0))

To evaluate our algorithms quantitatively and qualitatively, we perform both simulated phantom experiments and in-vivo experiments. The TV and TGV reconstruction algorithms are compared to commonly used methods like least-squares solution and single step frequency domain backprojection reconstruction. We show the superior results of the proposed reconstruction methods in terms of edge enhancement and noise reduction compared to standard methods.

10064-125, Session PSun

A machine learning-based approach to reflection artifact reduction in photoacoustic data

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Interventional applications of photoacoustic imaging often require visualization of point-like targets such as cross sectional needle and catheter tips. When these point-like targets are imaged in the presence of highly echogenic structures, the resulting acoustic wave often creates a reflection artifact that may appear as a true signal. We propose to use machine learning principles to identify these noise artifacts for removal. A convolutional neural network was trained to identify the location of 30,150 individual point targets from pre-beamformed data simulated with k-Wave (axial range: 3-20 mm, lateral range: 5-25 mm, speed of sound: 1440-1640 m/s, and radii: 1-5 mm). Based on 2,412 randomly selected test images, the mean axial and lateral point location errors were 0.28 mm and 0.37 mm, respectively. This trained network successfully identified the location of two point targets in a single image with mean axial and lateral errors of 2.6 mm and 2.1 mm, respectively. A true signal and a corresponding reflection artifact were then simulated. The same trained network identified the location of the artifact with mean axial and lateral errors of 2.1 and 3.0 mm, respectively. To differentiate the artifact from the true signal, the identified locations of these two wavefields were used as inputs to an independent k-Wave simulation, and the resulting wavefields were compared to those of the original image. Artifacts were rejected based on wavefront shape differences. These results demonstrate strong promise to simultaneously eliminate reflection artifacts from interventional images and identify point-like targets without requiring traditional geometry-based beamforming.

10064-126, Session PSun

Iterative photoacoustic image reconstruction for three-dimensional imaging by conventional linear-array detection with sparsity regularization

Hamid Moradi, Mohammad Honarvar, Shuo Tang, Septimiu E. Salcudean, The Univ. of British Columbia (Canada)

Iterative image reconstruction algorithms have the potential to reduce the computational time required for photoacoustic tomography (PAT). We have previously (SPIE Photonics West 2016) introduced a deconvolution-based photoacoustic reconstruction with sparsity regularization (DPARS). The method uses the sparsity regularization to solve the rank deficient systems caused by the limited angle of view and the directivity effect of conventional linear ultrasound transducers. These limitations are inevitable in many clinical applications such as prostate imaging. The direct method used in DPARS for solving the resulting system of equations has a very high computational cost, both in terms of memory and processing. To overcome the memory problem, an iterative solver such as a conjugate gradient method can be used in which the coefficient matrix does not need to be stored completely in memory. In addition, parallel processing can be used to reduce the computational time. We present in this paper a novel parallel computing approach to PAT that combines DPARS with the conjugate gradient method on Graphics Processing Units (GPUs). This novel approach, which we call iterative DPARS (iDPARS) enables us to reconstruct large 3D volumes (100?100?100) in less than 10 minutes.

Experimental and simulation data are used to compare the performance of the iterative inversion approach with conventional Delay-and-Sum (DAS) algorithm. By the use of limited measurements with conventional linear transducer arrays, we demonstrate that our approach provides PA images with higher Contrast-to-Noise Ratio and lower Root-Mean-Square errors.

10064-127, Session PSun

Free space ultrasound guided fluorescence diffuse optical tomography

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Fluorescence diffuse optical tomography (FDOT) is a noninvasive molecular imaging modality that reconstruct the fluorophore distribution from the surface light intensity distribution, which enables in preclinical small animal tumor studies. Incorporation of the prior information from structural image can ease the inherently ill-posed problem of FDOT. In this study, a free space ultrasound guided FDOT was demonstrated to provide both structural and functional information. The dual modality imaging system used a rotation gantry combined with an electron-multiplying charge-coupled device (EMCCD) and a 660 nm excitation laser source in trans-illumination mode to reconstruct the FDOT. The fluorescence data was collected from 16 projections along 360° range with integral time 500 ms per caption. The object was placed in the center of the gantry with the region of interest exposure in the air. The structural information and profile of the object were obtained by an ultrasound linear array transducer (Philips IU-22, L17-5, 6-17 MHz), which also provide the prior information to constrain the reconstruction of FDOT. To validate the performance of the proposed imaging system, phantoms and bio tissue experiment with Alexa 660 fluorophore inclusion inserted were conducted. The results show that the imaging system achieves accurate and has the potential for further in vivo study.

10064-128, Session PSun

Bayesian approach to image reconstruction in photoacoustic tomography

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In photoacoustic tomography, an initial acoustic pressure distribution created by an externally introduced light pulse is reconstructed from time-varying ultrasound measurements made on the surface of the object. In this work, this image reconstruction problem (inverse problem) is approached in Bayesian framework.

In the Bayesian approach, all parameters are modeled as random variables, which depend on each other through a model, and information about these parameters is expressed by probability distributions. In the inverse problem, the idea is to obtain information about the parameters of primary interest based on the measurements, the model, and the prior information about the parameters. In order to obtain computationally practical solutions, point estimates are computed to perform the image reconstruction. Furthermore, the reliability of the reconstructed images is assessed by computing the credibility intervals of the estimates.

The approach is investigated with numerical simulations in various imaging situations, including acoustically limited-view measurement setup. The results show that the Bayesian approach can be used to provide accurate estimates of the initial pressure distribution. Furthermore, it can give information about the uncertainty of the estimates, i.e. the reliability of reconstructed images. This may also benefit, for example, the design of the photoacoustic tomography measurement setup.

10064-130, Session PSun

Estimation and uncertainty quantification of optical properties directly from the photoacoustic time series

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Quantitative photoacoustic tomography seeks to estimate the optical parameters of a target given photoacoustic measurements as a data. Conventionally the problem is split in two steps: 1) the acoustical inverse problem of estimating the acoustic initial pressure distribution from the acoustical time series data; 2) the optical inverse problem of estimating the optical absorption and scattering from the initial pressure distributions. In this work, an approach for estimating the optical absorption and scattering directly from the acoustical time series is investigated with simulations.

The work combines a homogeneous acoustical forward model, based on Green's function solution of the wave equation, and a finite element method based diffusion approximation model of light propagation into a single forward model. This model maps the optical parameters of interest into a time domain signal. The model is used with a Bayesian approach to ill-posed inverse problems to form estimates of the posterior distributions for the parameters of interest. In addition to being able to provide point estimates of the parameters of interest, i.e. reconstruct the absorption and scattering distributions, the approach can be used to derive information on the uncertainty associated with the estimates.

10064-131, Session PSun

Software-based approach toward vendor-independent real-time photoacoustic imaging using ultrasound beamformed data

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Photoacoustic (PA) imaging has shown its potential for many clinical applications, but current research and usage of PA imaging are constrained by additional hardware costs to collect channel data, as the PA signals are incorrectly processed in existing clinical ultrasound systems. This problem arises from the fact that ultrasound systems beamform the PA signals as echoes from the ultrasound transducer instead of directly from illuminated sources. Consequently, conventional implementations of PA imaging include reliance on parallel channel acquisition from research platforms, which are not only slow and expensive, but are also mostly not approved by the FDA for clinical use. In previous studies, we have proposed synthetic-aperture based photoacoustic re-beamformer (SPARE) that uses ultrasound beamformed radio frequency (RF) data as input, which is readily available in clinical ultrasound scanners. The goal of the work is to implement SPARE beamformer in the clinical ultrasound system, and to experimentally demonstrate its real-time visualization. Assuming a high pulsed repetition frequency (PRF) laser is used, a PZT-based pseudo PA source transmission was synchronized with the ultrasound line trigger. As a result, the frame-rate increases as limiting the image field-of-view (FOV), and 50 frame and 20 frame per second were achieved for the FOV of 35 mm and 70 mm depth, respectively. Although in reality the maximum PRF of laser firing has a boundary, this result indicates the developed software is capable of displaying PA images with the maximum possible frame-rate for certain laser system without acquiring channel data.

10064-132, Session PSun

SNR enhancement for catheter based intravascular photoacoustic/ultrasound imaging

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Atherosclerosis, the most common cause of death, kills suddenly by arterial occlusion by thrombosis, which is caused by plaque rupture. Because a growing necrotic core is highly related to plaque rupture in atherosclerosis, distinguishing between fibrous plaque and lipid-rich plaque in real time is important, but has been challenging. Real-time photoacoustic imaging requires a pulse laser with high repetition rate, which tends to sacrifice pulse energy. Furthermore, a high repetition rate is hard to achieve at lipid-sensitive wavelengths, such as 1210 nm and 1720 nm. To address the unmet need, we have developed and tried the algorithm for PA imaging. We successfully acquired ex vivo PA images from the lipid cores of arterial plaques in rabbit arteries, using a low-power 1064-nm laser. PA images at were acquired with a custom-made catheter employing a single-element 40-MHz ultrasound transducer and a compact 1064-nm laser with the pulse energy of 5 ?J and the repetition rate of 24 kHz. Acquired raw data were processed in the time and frequency domains. In the time domain, a delay-and-sum algorithm was used for image enhancement. In the frequency domain, signals exceeding the MTF were removed. As a result, SNR was increased by about 10 dB without degrading spatial resolution. We were able to achieve high-speed and high-SNR lipid target imaging in animals in spite of the low lipid sensitivity of a 1064nm laser. These results show good promise for detecting lipid-rich plaques with a compact high-speed laser, which can be easily adapted for target clinical applications.

10064-26, Session 5

On design and characterization of a handheld annular array probe for volumetric optoacoustic/ultrasound imaging

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Hybridizing optoacoustic and ultrasound imaging comes with the promises of delivering the complementary morphological, functional and metabolic information of the investigating tissue. Despite many attempts, for volumetric imaging this integration remains challenging, mainly due to the essential differences in the physics of the imaging modalities. The present study is exploring the optimal geometrical properties for a hand-held probe, being capable of addressing the requirements of both imaging systems. In particular we are investigating the effect of size, number and geometrical location of the elements and their optimum configuration. In order to assess and optimize the performance characteristics of both imaging systems, series of analysis in the frameworks of system transfer matrix and final image were carried out. The former has been evaluated by means of voxel crosstalk and eigen-analysis, revealing information about the spatial

sensitivity, aliasing and ability of focusing on specific target. The latter investigates the feature fidelity to the investigating object and is associated with the merits of reconstruction algorithm. To avoid biased interpretations, two image reconstruction approaches, namely weighted SAFT and model based were applied on the simulated and experimental data. The simulation results suggest that the annular-spiral array consist of 128 elements of 3 mm radius operating at 5 MHz central frequency showcases a good agreement with the physical requirements of both imaging systems. These results are further validated experimentally, by scanning a mono-element in the same fashion as the designed array configurations. We believe that our design facilitates the transformation from bench to bedside.

10064-27, Session 5

Simultaneous in vivo imaging of diffuse optical reflectance, optoacoustic pressure and ultrasonic scattering

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We will present reflection-mode bioimaging system providing complementary optical, photoacsoutic and acoustic measurements by acoustic detector after each laser pulse with 2kHz repetition rate. The photons absorbed within the biological tissue provide optoacoustic (OA) signals, the photons absorbed by the external electrode of a detector provide the measurable diffuse reflectance (DR) from the sample and the probing ultrasonic (US) pulse.

To demonstrate the in vivo capabilities of the system we performed complementary DR/OA/US imaging of small laboratory animals and human palm with 3.5mm/50?m/35?m lateral resolution at up to 3 mm diagnostic depth. Functional OA and DR imaging demonstrated the levels of tissue vascularization and blood supply. Structural US imaging was essential for understanding the position of vessels and zones with different perfusion.

Before BiOS-2017 we plan to accomplish more in vivo experiments validating the developed triple-modality system as diagnostic tool to detect vascularization as well as mechanisms of vascular changes when monitoring response to therapy.

10064-28, Session 5

Real-time intravascular photoacoustic/ ultrasound imaging of lipid-laden plaque at 20-frames per second

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Intravascular photoacoustic/ultrasound (IVPA/US) imaging is an emerging hybrid modality for advanced assessment of plaque vulnerability by simultaneously providing morphological and lipid-specific chemical information of the artery wall with a ~6 mm imaging depth. Thus far, clinical translation of this technology is still hampered by the lack of a real-time IVPA/US system with a clinically relevant imaging speed. Here, we demonstrate a compact IVPA/US system capable of imaging at 20 frame/sec, 1.7 micron excitation, and with a different A-line strategy. In our system, a custom-built 2-kHz master oscillator power amplifier (MOPA)-pumped optical parametric oscillator with a maximum output pulse energy of 1 mJ at 1725 nm was used as the optical excitation source. A different A-line strategy for IVPA and IVUS imaging was demonstrated as 100 and 200 A-lines per frame, respectively. This approach balanced the trade-off between the number of A-lines per frame and the imaging speed and further maximized the IVPA/US imaging functionality. The 20-frame/sec real-time imaging speed was achieved by a fast online imaging processing and display algorithm. The performance of the imaging system, including spatial resolution, sensitivity, and specificity was evaluated by standard phantoms with a high-sensitivity collinearly designed IVPA/US catheter. Its imaging functionality was compared with the case at low imaging speed and high number of A-lines per frame. The clinical utility of this system was further evaluated by ex vivo imaging of an excised fresh human coronary artery and comparison with the gold standard histology. These results collectively put our system one step closer to in vivo imaging.

10064-29, Session 5

Photoacoustic and laser-ultrasound imaging of arterial tissue ex-vivo

Jami L. Johnson, Kasper van Wijk, Mervyn Merrilees, The Univ. of Auckland (New Zealand)

Arterial tissue imaging and characterization is important for disease diagnosis, treatment planning and monitoring, and research into disease processes. The high optical contrast of photoacoustic imaging can distinguish molecules with unique optical spectra from surrounding arterial tissue, while ultrasound is sensitive to variations in acoustic properties. Combining photoacoustics with ultrasonics provides more comprehensive diagnostic information by extracting molecular information from photoacoustics and structural information from ultrasound. Furthermore, ultrasound may be able to distinguish molecules with indistinct optical spectra but strong acoustic properties, such as calcification. In this work we will present our results applying our recently developed all-optical, multi-channel photoacoustic and laser-ultrasound imaging techniques to arterial tissue ex-vivo. We first apply redatuming techniques to remove reverberation artifacts, and subsequently image with time-reversal.

10064-30, Session 5

Three-dimensional printed ultrasound and photoacoustic training phantoms for vasculature access

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Ultrasound (US) imaging is widely used to guide vascular access procedures such as arterial and venous cannulation. As needle visualisation with US imaging can be very challenging, it is easy to misplace the needle in the patient and it can be life threating. Photoacoustic (PA) imaging is well suited to image medical needles and catheters that are commonly used for vascular access. To improve the success rate, a certain level of proficiency is required that can be gained through extensive practice on phantoms. Unfortunately, commercial training phantoms are expensive and custom-made phantoms usually do not replicate the anatomy very well. Thus, there is a great demand for more realistic and affordable ultrasound and photoacoustic imaging phantoms for vasculature access procedures training. Three-dimensional (3D) printing can help create models that replicate complex anatomical geometries. However, the available 3D printed materials do not possess realistic tissue properties. Alternatively, tissue-mimicking materials can be employed using casting and 3D printed moulds but this approach is limited to the creation of realistic outer shapes with no replication of complex internal structures. In this study, we developed a realistic vasculature access phantom using a combination of mineral oil based materials as background tissue and a non-toxic, water dissolvable filament material to create complex vascular structure using 3D printing.

US and PA images of the phantoms comprising the complex vasculature network were acquired. The results show that 3D printing can facilitate the fabrication of anatomically realistic training phantoms, with designs that can be customized and shared electronically.

10064-31, Session 5

Dual-modality 3D photoacoustic and speed of sound imaging with optical ultrasound detection

Robert Nuster, Gerhild Wurzinger, Guenther Paltauf, Karl-Franzens-Univ. Graz (Austria)

Recently, we have shown that CCD camera based visualization of sound fields is a promising alternative detection approach for fast, high resolution 3D photoacoustic imaging (PAI). To fully exploit the potential of this detection approach, to gain image resolution in the range of 30µm, it is necessary to incorporate heterogeneities of the speed of sound (SOS) in the image reconstruction algorithm. Hence, in the proposed work the idea and the concept are shown how speed of sound imaging can be added to the camera based PAI setup to obtain dual-modality, perfectly co-registered 3D photoacoustic and SOS images.

In detail, the camera based optical phase contrast ultrasound detection setup for PAI is extended by an array of external stair-shaped absorbers used as laser ultrasound (LUS) sources for SOS imaging. A LUS background image (without sample) shows parallel lines, indicating wave fronts generated by the absorbers. Each of the wave fronts has traversed the sample at a different distance to the camera. Acoustic heterogeneities within the acoustic ray path cause a bending of the recorded line structure. The amount of the bending corresponds to the variation of the average speed of sound and is analyzed for SOS reconstruction. Since the setup records projections of the acoustic fields the reconstruction of the 3D photoacoustic and SOS images requires to apply the inverse Radon transform to the obtained projection data while rotating the sample. The initial photoacoustic projection data are obtained using the two dimensional time-reversal reconstruction algorithm taking into account the SOS heterogeneities of the sample. Simulations and first experimental results are shown.

10064-32, Session 5

Highly specific spectroscopic photoacoustic molecular imaging of dynamic optical absorption shifts of an antibody-indocyanine green contrast agent

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Improved techniques for breast cancer screening are critically needed as current methods lack diagnostic accuracy. Using spectroscopic photoacoustic (sPA) molecular imaging with a priori knowledge of optical absorption spectra allows suppression of endogenous background signal, increasing the overall sensitivity and specificity of the modality to exogenous contrast agents. Here, sPA imaging was used to monitor antibody-indocyanine green (ICG) conjugates as they undergo optical absorption spectrum shifts after cellular endocytosis and degradation to allow differentiation between normal murine mammary glands from breast cancer by enhancing molecular imaging signal from target (B7-H3)-bound antibody-ICG. First, B7-H3 was shown to have highly specific (AUC of 0.93) expression on both vascular endothelium and tumor stroma in malignant lesions through quantitative immunohistochemical staining of B7-H3 on 279 human samples (normal (n=53), benign lesions (11 subtypes, n=182), breast cancers (4 subtypes, n=97)), making B7-H3 a promising target for sPA imaging. Second, absorption spectra of intracellular and degraded B7-H3-ICG and Isotype control (Iso-ICG) were characterized through in vitro and in vivo experiments. Finally, a transgenic murine breast cancer model (FVB/N-Tg(MMTVPyMT)634Mul) was imaged, and sPA imaging in found a 3.01 (IQR 2.63, 3.38, P<0.001) fold increase in molecular B7-H3-ICG signal in tumors (n=80) compared to control conditions (B7-H3-ICG in tumor negative animals (n=60), Iso-ICG (n=30), blocking B7-H3+B7-H3-ICG (n=20), and free ICG (n=20)) despite significant tumor accumulation of Iso-ICG, confirmed through ex vivo histology. Overall, leveraging anti-B7-H3 antibody-ICG contrast agents, which have dynamic optical absorption spectra representative of molecular interactions, allows for highly specific sPA imaging of murine breast cancer.

10064-33, Session 5

Glycol-chitosan-coated gold nanoparticles for photoacoustic imaging of lymph nodes

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A key step in staging cancer is the diagnosis of metastasis that spreads through lymphatic system. For this reason, researchers develop various methods of sentinel lymph node mapping that often use a radioactive tracer. This study introduces a safe, cost-effective, high-resolution, high-sensitivity, and real-time method of visualizing the sentinel lymph node: ultrasound-guided photoacoustic (US/PA) imaging augmented by a contrast agent. In this work, we use clearable gold nanoparticles covered by a biocompatible polymer (glycol chitosan) to enhance cellular uptake by macrophages abundant in lymph nodes. We incubate macrophages with glycol-chitosan-coated gold nanoparticles (0.05 mg Au/ml), and then fix them with paraformaldehyde solution for an analysis of in vitro dark-field microscopy and cell phantom. The analysis shows enhanced cellular uptake of nanoparticles by macrophages and strong photoacoustic signal from labeled cells in tissue-mimicking cell phantoms consisting gelatin solution (6 %) with silica gel (25 ?m, 0.3%) and fixed macrophages. The in-vivo US/PA imaging of cervical lymph nodes in healthy mice (nu/nu, female, 5 weeks) indicates a strong photoacoustic signal from a lymph node 10 minutes post-injection (2.5 mg Au/ml, 80 ?l). The signal intensity and the nanoparticle-labeled volume of tissue within the lymph node continues to increase until 4 h post-injection. Histological analysis further confirms the accumulation of gold nanoparticles within the lymph nodes. This work suggests the feasibility of molecular/cellular US/PA imaging with biocompatible gold nanoparticles as a photoacoustic contrast agent in the diagnosis of lymph-node-related diseases.

10064-34, Session 6

Imaging of post-embryonic stage model-organisms at high resolution using multi orientation optoacoustic mesoscopy

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Model organisms such as zebrafish play an important role for developmental biologists and experimental geneticists. Still, as they grow into their post?embryonic stage of development it becomes more and more difficult to image them because of high light scattering inside biological tissue. Optoacoustic mesoscopy based on spherically focused, high frequency, ultrasound detectors offers an alternative, where it relies on the focusing capabilities of the ultrasound detectors in generating the image rather than on the focusing of light. Nonetheless, because of the limited numerical aperture the resolution is not isotropic, and many structures, especially elongated ones, such as blood vessels and other organs, are either invisible, or not clearly identifiable on the final image. Herein, based on high frequency ultrasound detectors at 100 MHz and 50 MHz we introduce multi orientation (view) optoacoustic mesoscopy. We collect a rich amount of signals from multiple directions and combine them using a weighted sum in the Fourier domain and a Wiener deconvolution into a single high resolution three-dimensional image. The new system achieves isotropic resolutions on the order of 10 μm in-plane, 40 μm axially, and SNR enhancement of 15 dB compared to the single orientation case. To showcase the system we imaged a juvenile zebrafish ex vivo, which is too large to image using optical microscopic techniques, the reconstructed images show unprecedented performance in terms of SNR, resolution, and clarity of the observed structures. Using the system we see the inner organs of the zebrafish, the pigmentation, and the vessels with unprecedented clarity.

10064-35, Session 6

All-optical side-viewing pulse-echo ultrasound probe for intravascular imaging

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High frequency ultrasound probes such as intravascular ultrasound (IVUS) and intracardiac echocardiography (ICE) catheters can be invaluable for guiding minimally invasive medical procedures in cardiology such as coronary stent placement and ablation. With current-generation ultrasound probes, ultrasound is generated and received electrically. The complexities involved with fabricating these electrical probes can result in high costs that limit their clinical applicability. Additionally, it can be challenging to achieve wide transmission bandwidths and adequate wideband reception sensitivity with small piezoelectric elements. Optical methods for transmitting and receiving ultrasound are emerging as alternatives to their electrical counterparts. They offer several distinguishing advantages, including the potential to generate and detect the broadband ultrasound fields (tens of MHz) required for high resolution imaging. In this study, we developed a miniature, side-viewing, pulse-echo ultrasound probe for intravascular imaging, with fibre-optic transmission and reception. The axial resolution was better than 70 microns, and the imaging depth in tissue was greater than 1 cm. Ultrasound transmission was performed by photoacoustic excitation of a carbon nanotube/polydimethylsiloxane nanocomposite; ultrasound reception, with a fibre-optic Fabry-Perot cavity. Ex vivo tissue studies, which included healthy swine tissue and diseased human tissue, demonstrated the strong potential of this technique. To our knowledge, this is the first study to achieve an all-optical pulse-echo ultrasound probe for intravascular imaging. The potential for performing all-optical B-mode imaging (2D and 3D) with virtual arrays of transmit/receive elements, and hybrid imaging with pulse-echo ultrasound and photoacoustic sensing are discussed.

10064-36, Session 6

New photoacoustic platform for early detection of circulating clots to prevent stroke and other fatal thromboembolic complications

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Nearly 800,000 people in the U.S. experience an incident of stroke each year; ~80% of these are first time occurrences and ~87% are ischemic in nature. Someone dies of a stroke every few minutes in the U.S. but despite its prevalence there have been minimal advances in the early detection and screening of thromboembolic events, especially during patient post-operative periods or in genetically predisposed individuals. Environmental or genetic factors may disrupt the balance between coagulation and lysis of micro-thrombi in circulation and increase the risk of stroke. We introduced here a novel in vivo multicolor negative-contrast photoacoustic (PA) flow cytometry (PAFC) platform with many innovations including customized high pulse repetition rate 1064 laser from IPG Photonics Corporation, powerful laser diode array, multichannel optical schematic, and time-resolved recording system. Using animal models, we verified the potential of this technology to detect small clots in relatively large vessels in vivo. If future clinical trials using a cost-effective, easy-to-use, safe, watch-like, wearable PA probe are successful, PAFC could provide breakthroughs in early monitoring of the growth in size and number of small clots that may predict and potentially prevent fatal thromboembolic complications. We also believe that this technology could be utilized to assess therapeutic benefits of anticoagulants and develop more efficient dosage in treatments by analyzing changes in the composition and frequency of micro-thrombi

10064-37, Session 6

Microstructured polymer optical fiber sensors for optoacoustic endoscopy

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In the last two decades intravascular ultrasound (IVUS) arise as an important imaging method to visualize atheromatous plaque in the coronary arteries. Vulnerable atherosclerotic plaques (VP) are characterized by the presence a lipid-rich necrotic core, which is soft and mechanically unstable, covered by a thin fibrous cap, which is weakened by inflammation. The rupture of these VP releases the thrombogenic contents of the plaque into the bloodstream resulting in acute myocardial infarction or in a stroke with a high mortality rate. Such events cause the majority of acute cardiovascular events and sudden cardiac deaths, resulting in 17 million fatalities worldwide annually, representing 29% of all global deaths.

An IVUS catheter contains at its tip a transducer or an array of transducers which emits ultrasonic waves and receives the backscattered signal from the tissue. The tomographic views generated allows an accurate determination of location and morphology of atherosclerotic plaque but has a limited specificity for different soft tissue types and therefore to discriminate the plaque composition. Intravascular photoacoustics (IVPA) (also known as optoacoustics), can complement IVUS giving spectroscopic information by mapping the optical absorption distribution. In order to achieve a practical IVUS/IVPA catheter is mandatory to have a wideband ultrasonic detector with enough sensitivity despite the necessary miniaturization to be fitted in less than 1mm to pass through thin vasculature.

Optical sensors based on optical fibers have a form factor specially well suited for IVPA applications, where wire-like shaped transducers are required with good lateral sensitivity and ultra-wide ultrasonic detection bandwidth . Optical fiber sensors have better sensitivity per unit of area and large detection bandwidth than traditional transducers based on piezoelectric materials when the miniaturization of the transducer is taken into account. Moreover, the optical fiber sensors provide immunity to

electrical perturbations. Our group demonstrated that ultrasonic sensitivity of an interferometric single mode polymer optical fiber sensor is more than an order of magnitude greater than a silica counterpart. Single mode fiber operation is mandatory for high performance interferometric sensors or for FBG sensors (fiber Bragg gratings). Polymer optical fibers currently can be made endlessly single-mode by means of a microstructured. FBGs can be inscribed in this kind of microstructured single mode optical fibers. The sensitivity region of a FBG based sensor is located in the grating structure, whereas in the interferometric optical fiber sensor it is distributed along the acoustic interaction length.

In this paper, we compare the performance of two interferometric mPOF sensors based on PMMA and humidity insensitive polymer TOPAS and two FBG sensors inscribed in PMMA and TOPAS mPOF for intravascular optoacoustic endoscope. This will be done based on the comparison of sensitivity, dynamic range, bandwidth, spatial resolution and compactness.

10064-38, Session 6

Assessment of plaque vulnerability in atherosclerosis via photoacoustic imaging of targeted liposomal ICG J-aggregates

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While molecular and cellular imaging can be used to visualize the conventional morphology characteristics of vulnerable plaques, there is a need to monitor other physiological factors correlated with high rupture rates; a high M1 activated macrophage concentration is one such indicator of high plaque vulnerability. Here, we present a molecularly targeted contrast agent for intravascular photoacoustic (IVPA) imaging consisting of liposomes loaded with indocyanine green (ICG) J-aggregates with high absorption at 890 nm, allowing for imaging in the presence of blood. This “Lipo-ICG” was targeted to a biomarker of M1 activated macrophages in vulnerable plaques: folate receptor beta (FR?). The targeted liposomes accumulate in plaques through areas of endothelial dysfunction, while the liposome encapsulation prevents nonspecific interaction with lipids and endothelium. Lipo-ICG specifically interacts with M1 activated macrophages, causing a spectral shift and change in the 890/780 nm photoacoustic intensity ratio upon breakdown of J-aggregates. This sensing mechanism enables assessment of the M1 activated macrophage concentration, providing a measure of plaque vulnerability. In a pilot in vivo study utilizing ApoE deficient mouse models of atherosclerosis, diseased mice showed increased uptake of FR? targeted Lipo-ICG in the heart and arteries vs. normal mice. Likewise, targeted Lipo-ICG showed increased uptake vs. two non-targeted controls. Thus, we successfully synthesized a contrast agent to detect M1 activated macrophages in high risk atherosclerotic plaques and exhibited targeting both in vitro and in vivo. This biocompatible agent could enable M1 macrophage detection, allowing better clinical decision making in treatment of atherosclerosis.

10064-39, Session 6

In vivo multimodal photoacoustic-fluorescence monitoring of cancer-induced circulating exosomes

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Approximately, 90% of cancer-related deaths are due to metastases. Detection of circulating tumor cells (CTCs) has shown to be a potential prognostic marker for metastatic development and therapeutic efficacy. However, with the current ex vivo CTC detection assays, deadly metastases have already developed. Therefore, new approaches for the early detection of metastatic disease are greatly needed. Apart from CTCs, circulating tumor-associated particles (CTPs) which include exosomes, are released from primary tumor cells in greater amounts long before the appearance of CTCs in circulation. Because of this, CTPs have the potential to be a valuable biomarker for early metastatic disease. However, little progress has been made in the high sensitivity detection of CTPs, especially, in vivo. Here, we present a new technical platform that shows evidence that in vivo integrated photoacoustic (PA) and fluorescence flow cytometry (PAFFC) techniques can be used for the detection of single CTPs in melanoma and breast cancer. Using intrinsic melanin, dyes, and genetically modified cells with fluorescent proteins as PAFFC contrast agents, we are able to detect single CTPs in circulation released from melanoma and breast tumor-bearing mice. The two-beam, time-of-flight, PAFFC can also measure the sizes of CTPs, bulk and rolling CTCs, and CTC clusters. Each of these have a unique signal, producing narrow, medium, and wide signal peaks, respectively, with no influence of blood flow instability. Because many CTPs can be released by a single tumor cell, and in vivo PAFFC can examine the whole blood volume, the proposed diagnostic platform has the potential for dramatic high sensitivity improvement (up to 10^5 – fold) early cancer diagnosis compared to current ex vivo CTC assays being used.

10064-40, Session 7

Three-dimensional photoacoustic tomography and inversion for accurate quantification of chromophore distributions

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Obtaining quantitatively accurate, high-resolution, estimates of chromophore distributions in 3D is the holy grail of photoacoustic tomography. It is a challenging problem that requires a non-linear optical inversion to deal with the fluence-related spatial and spectral distortion. High-dimensional, model-based, minimisation strategies achieve optimal results in simulation but have not been convincingly demonstrated experimentally.

A meticulous and rigorous experimental study was conducted to show that 3D high-resolution chromophore quantification can be achieved: a crucial step towards in vivo implementation. A quasi-Newton optimisation using a diffusion model of light transport was employed. To provide additional robustness to noise and experimental uncertainty, we further tested a reconstruction-classification approach that employs a probabilistic model to describe the optical properties as a finite number of optically distinct classes.

The phantom consisted of four 580?m diameter tubes with different ratios of copper and nickel sulphate as hemoglobin analogues, submersed in a background of intralipid and india ink. For all components, the absorption, scattering, photostability and Grüneisen parameter were characterised independently.

A V-shaped pre-clinical imaging scanner was used, enabling 3D imaging at the high resolution, good sensitivity, and wide bandwidth characteristic of Fabry-Perot sensors. The optical beam profile and position were determined experimentally. Nine wavelengths between 750 and 1110 nm were used. Iterative time-reversal acoustic reconstruction yielded high quality initial pressure images for an 18x17x13 mm3 volume at sub-100?m resolution.

The model-based optimisation strategies, which also accounted for the variation in Grüneisen parameter, were then used to obtain successful chromophore concentration estimates. Performing the imaging and the reconstructions both fully in 3D makes accurate quantification possible.

10064-41, Session 7

Experimental validation of a Monte-Carlo-based inversion scheme for 3-D quantitative photoacoustic tomography

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Quantitative photoacoustic tomography (qPAT) aims to extract physiological parameters, such as blood oxygen saturation (sO2), from measured multiwavelength image data sets. The challenge of this approach lies in the inherently nonlinear fluence distribution in the tissue, which has to be accounted for by using an appropriate model, and the large scale of the inverse problem. In addition, the accuracy of experimental and scanner-specific parameters, such as the wavelength dependence of the incident fluence, the acoustic detector response, the beam profile and divergence, needs to be considered. This study aims at quantitative imaging of blood sO2, as it has been shown to be a more robust parameter compared to absolute concentrations. We propose a Monte-Carlo-based inversion scheme in conjunction with a reduction in the number of variables achieved using image segmentation. The inversion scheme is experimentally validated in tissue-mimicking phantoms consisting of polymer tubes suspended in a scattering liquid. The tubes were filled with chromophore solutions at different concentration ratios. 3-D multispectral image data sets were acquired using a Fabry-Perot based PA scanner. The effect of calibrated absorbers in the PA images on the convergence of the inversion was evaluated. A quantitative comparison of the measured data with the output of the forward model is presented. The capability of this approach to recover 3-D maps of absolute concentration ratios, i.e. blood sO2, and chromophore concentrations, is evaluated. The accuracy and resolution of the determined parameters given the detection noise is discussed.

10064-42, Session 7

Ultrasound spectral analysis of photoacoustic signals from red blood cell populations at different optical wavelengths

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Ultrasound (US) spectral analysis of photoacoustic (PA) signals is an emerging technique that analyze the US power spectrum of PA signals to quantify tissue microstructures. PA spectral analysis has been correlated to changes in the size, morphology and concentration of absorbers in a sample smaller than the system resolution. However, the calculated spectral parameters are still not system independent due to difficulty in eliminating light distribution for different optical wavelengths. Changes in US spectral parameters for different optical wavelengths need to be examined.

A gelatin vessel phantom is used. The vessels contain red blood cells comprised of oxy, deoxy and methemoglobin induced using sodium citrate, sodium hydrosulfite and sodium nitrite, respectively. The samples were imaged using the VevoLAZR system at wavelengths 680 – 910nm with 10nm step size. The radiofrequency (RF) signals were analyzed to calculate the spectral slope. The results were compared to simulated RF signals acquired using the mcxyz Monte Carlo package coupled to the solution of the PA wave equation using the green function approach.

Changes in the spectral slope as a function of optical wavelength was detected. For longer optical wavelengths, the spectral slope increased from -0.36 to -0.31dB/MHz for deoxyhemoglobin, and decreased from -0.25 to -0.32dB/MHz for oxyhemoglobin and from -0.24 to -0.32dB/MHz for methemoglobin. This was correlated to changes in the fluence distribution as optical properties change for different wavelengths. The correlation of the spectral slope to the chromophore content can be used beside spectral unmixing for better estimation of hemoglobin content.

10064-43, Session 7

Visualization/quantification of HER2 expression in human breast cancer xenografts with 800CW-pertuzumab using acousto-optically assisted fluence compensated photoacoustic imaging

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Light fluence inside turbid media can be experimentally mapped by measuring ultrasonically modulated light (Acousto-optics). To demonstrate the feasibility of fluence corrected Photoacoustic (PA) imaging, we have realized a tri-modality (i.e. photoacoustic, acousto-optic and ultrasound) tomographic small animal imaging system. Wherein PA imaging provides high resolution map of absorbed optical energy density, Acousto-optics yields the fluence distribution map in the corresponding PA imaging plane and Ultrasound provides morphological information. Further, normalization of the PA image with the acousto-optically measured fluence map results in an image that directly represents the optical absorption.

Human epidermal growth factor receptor 2 (HER2) is commonly found overexpressed in human cancers, among which breast cancers, resulting in a more aggressive tumor phenotype. Identification of HER2-expression is clinically relevant, because cancers overexpressing this marker are amenable to HER2-directed therapies, among which antibodies trastuzumab and pertuzumab. Here, we investigate the feasibility and advantage of acousto-optically assisted fluence compensated PA imaging over PA imaging alone in visualizing and quantifying HER2 expression. For this experiment, nude mice were xenografted with human breast cancer cell lines SKBR3 and BT474 (both HER2 overexpressing), as well as HER2-negative MDA-MB-231. To visualize HER2 expression in these mice, HER2 monoclonal antibody pertuzumab (Perjeta®, Roche), was conjugated to near-infrared dye IRDye 800CW (800CW, LICOR Biosciences) at a ratio of 1?2 antibody to 800CW. When xenograft tumors measured ≥ 100 mm3, mice received 100 μg 800CW-pertuzumab intravenously. Three days post injection, mice were scanned for fluorescence signal with an IVIS scanner. After fluorescence scans, mice were euthanized and imaged in our PA tomographic imaging system.

10064-44, Session 7

Exploiting statistical independence for quantitative photoacoustic tomography

Lu An, Benjamin T. Cox, Univ. College London (United Kingdom)

To unlock the full capability of photoacoustic tomography as a quantitative, high resolution, molecular imaging modality, the problem of quantitative photoacoustic tomography must be solved. The aim in this is to extract

clinically relevant functional information from photoacoustic images by finding the concentrations of the chromophores in the tissue. This is a challenging task due to the effect of the unknown but spatially and spectrally varying fluence within the tissue. Many inversion schemes that include a model of the fluence have been proposed, but these have yet to make an impact in pre-clinical or clinical imaging.

In this study, we have exploited the statistical independence (or dependence) of the chromophores to improve the robustness and hence the usefulness of the model-based inversion methods. This was achieved by minimising mutual information terms in addition to the least squares data error within a gradient-based optimisation scheme. More accurate quantification and faster convergence can be achieved in the presence of experimental uncertainties, because, unlike the data error, the mutual information is not affected by the errors in the forward modelling.

Experimental studies based on tube phantoms, as well as supporting numerical simulations, show that incorporating the statistical independence significantly improves the performance of model-based inversion. Finally, we demonstrate the applicability for in vivo images by quantifying the concentration of polymer nanoparticles injected at a tumour region in a mouse flank.

10064-45, Session 7

20 frames per second model-based reconstruction in cross-sectional optoacoustic tomography

Lu Ding, Xosé Luís Deán-Ben, Daniel Razansky, Helmholtz Zentrum München GmbH (Germany)

When it comes to real-time image rendering and visualization in optoacoustic tomography, the simple analytical back-projection inversion formula becomes the ultimate choice due to its simplicity and low computational complexity. On the other hand, model-based reconstruction algorithms are generally known to render better image quality and accuracy due to their ability to model arbitrary detection geometries, transducer shapes and other experimental factors. However, model-based inversion is associated with high computational complexity and long reconstruction times due to large-scale matrix vector multiplications, thus cannot be employed for real-time image rendering. Herein, we introduce a model-based reconstruction method based on on-the-fly calculation of the model-matrix combined with a new discretization approach for the calculation of the model matrix. The new methodology enables an efficient parallel implementation on a graphics processing unit (GPU) with extremely low memory overhead. With a few simplifications, we show that the important characteristics of the model matrix can be stored in a small look-up table, reducing the complexity of the on-the-fly calculations to a few basic mathematical operations. We demonstrate that the new method achieves real-time model-based inversion in cross-sectional optoacoustic tomography without sacrificing image quality.

10064-46, Session 8

Non-negative constrained inversion approaches for unmixing chromophores in multispectral optoacoustic tomography

Lu Ding, Xosé Luís Deán-Ben, Daniel Razansky, Helmholtz Zentrum München GmbH (Germany)

Multispectral optoacoustic tomography (MSOT) renders the distribution of spectrally-distinctive optical absorbers deep inside scattering tissues. Images representing the distribution of absorbing substances are commonly obtained via a two-step procedure. First, the optical absorption distribution within the tissue is separately reconstructed for each illumination wavelength. Subsequently, contributions of the different absorbers (chromophores) are separated via a multispectral unmixing algorithm. Due to experimental and modeling imperfections, negative values often appear in both the reconstructed optical absorption images and the spectrally unmixed images. Since negative values have no physical meaning, accuracy and quantitativeness can potentially be improved by imposing non-negativity constraints on the reconstruction and unmixing steps. With these additional constraints, the reconstruction and unmixing problems can no longer be considered linear transformations, thus the final result greatly depends on the particular model employed and on the constraint imposed. Herein, we compare several non-negative constrained approaches where reconstruction and unmixing can be either combined in a single inverse step or solved as separate problems. The results are further compared to the equivalent unconstrained approaches. The quantitative reconstruction performance and sensitivity of the different methods in detecting small chromophore concentrations is tested in tissue-mimicking phantoms and with real mouse imaging data. The results obtained allow establishing the most efficient methods for resolving the spatial distribution of spectrally-distinct absorbing substances.

10064-47, Session 8

Optoacoustic endoscopy with optical and acoustic resolution

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Optoacoustic endoscopes operating on ultrasonic diffraction limitations using focused ultrasound detectors, achieve lateral resolution spanning from hundreds to tens of micro-meters with an imaging depth up to several millimeters. Such acoustic resolution optoacoustic endoscopes (AR-OE) have been showcased to provide high resolution visualization of the intestinal vasculature of small animals in vivo. Even higher resolution can be achieved by optical-resolution optoacoustic endoscopy (OR-OE) using a focused laser beam, in analogy to intra-vital optical microscopy. However, as intra-vital microscopy, OR-OE is affected by light scattering in tissue and thus limited to superficial structures. Herein, we propose an optical resolution and acoustic resolution optoacoustic endoscope appropriate for improving the endoscopic depth and resolution range. The data presented herein for the first time to our knowledge demonstrate the feasibility of hybrid optical resolution and acoustic resolution optoacoustic endoscopy with a single sensor. The probe has a diameter of 3.6 mm, which is compatible with the working channel of white-light optical endoscopes. As shown, by focusing the laser light with the GRIN fiber, an optical resolution of the order of 13 μm can be achieved, with an impressive SNR of 20 based on the characterization of a 10 μm diameter suture with the laser energy below the ANSI safety limit. The results of the phantoms and mouse ear measurements ex vivo show that proposed herein hybrid endoscopy system can gain optical resolution imaging of the surface and tomography imaging for the deeper features.

10064-49, Session 8

Non-invasive volumetric optoacoustic imaging of cardiac cycles in acute myocardial infarction model in real-time

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Extraction of murine cardiac functional parameters on a beat-by-beat basis remains challenging with the existing imaging modalities, such as small

animal MRI, CT or Ultrasound, due to their insufficient temporal resolution when acquiring 3D images.

We present a new approach for the evaluation of functional cardiac parameters in living mice based on analyzing high-contrast volumetric optoacoustic (OA) images acquired at video rate without using cardiac gating. The imaging system consists of 512-element spherical ultrasound array centered at 5MHz and covering a solid angle of 140º, which can capture 3D image data at a frame rate of up to 100Hz limited by the pulse repetition frequency of the laser. The new technique is applied to studying the functional parameters of cardiac cycles in murine model of acute myocardial infarction. With the establishment of a suitable infarct mouse model for cardiac optoacoustic imaging, blood-pool contrast injection events were monitored in real-time with the imaging system capable of consistently resolving important cardiac anatomy and function.

Utilizing frequency-based analysis, cardiac muscles were segmented with a robust automated method, enabling calculations of the pulmonary transit time. Infarcted hearts can be clearly differentiated from healthy controls based on the pulmonary transit time, while no statistically significant difference is observed in the heartbeat. Complementary analysis was done by magnetic resonance imaging (MRI) for validation of decreased heart function in infarct models. The pulmonary transit time was then confirmed to be positively correlated to the infarct size, characterized as the percentage of infarcted myocardium compared to the entire myocardium, and an inverse relationship was found with the ejection fraction.

The new imaging approach eliminates the need for cardiac gating and pseudo-real-time acquisition of images over multiple cardiac cycles to eliminate motion artifacts. As a result, the ability to render true volumetric data from a beating heart with high spatial-temporal resolution opens new prospects for non-invasive characterization of cardiac function during the progression of disease, and assessment of efficacy of therapies.

10064-50, Session 8

Real-time photoacoustic flow cytography and photothermolysis of single circulating melanoma cells in vivo

Yun He, Lidai Wang, Junhui Shi, Junjie Yao, Lei Li, Ruiying Zhang, Washington Univ. in St. Louis (United States); Chih-Hsien Huang, Jun Zou, Texas A&M Univ. (United States); Lihong V. Wang, Washington Univ. in St. Louis (United States)

Melanoma is the deadliest type of skin cancer, primarily due to a high propensity for metastasis. Since dissemination of circulating tumor cells (CTCs) is melanoma's major route of metastasis, detection and destruction of CTCs can impede metastasis and improve patient prognosis. Extensive studies employing exogenous agents to detect the tumor-specific biomarkers and guide therapeutics to CTCs have achieved promising results, but the biosafety remains a critical concern.

Taking another approach, physical detection and destruction of CTCs is a more direct and safe way to reduce metastasis risks. Melanoma cells have a high melanosome expression, providing a striking absorption contrast in the red to near-infrared spectrum. Exploiting this endogenous contrast, we report high-resolution CTC imaging, using our new dual-wavelength PA flow cytography, in combination with real-time CTC photothermolysis by pinpoint lethal irradiation from a therapy laser.

To image CTCs on the fly, our system employs a 500-kHz short-pulsed imaging laser that works at both 532 nm and 1064 nm wavelengths and a 500-Hz-resonance-frequency scanning mirror, achieving a 10 Hz 3D volumetric frame rate over a 2 ? 0.5 mm2 area. The therapy laser is triggered, by real-time hardware, immediately after flow cytography detects a CTC to lethally irradiate it in a thermally confined manner. We have successfully imaged single CTCs in mouse trunk vessels with a contrast-to-noise ratio of ~25 and performed real-time CTC photothermolysis. A pseudo-therapy study demonstrated the performance and the potential clinical value of our method, which can facilitate treatment of metastasis by clearing CTCs from vasculature.

10064-51, Session 8

Whole-body preclinical imaging with a multi-view Fabry Perot scanner

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Planar Fabry-Pérot (FP) ultrasound sensor arrays have been used to produce in-vivo photoacoustic images of exquisite quality due to their broad detection bandwidth and dense spatial sampling. Single-plane and V-shaped FP scanners have been used to image with high resolution to depths of <1cm. In this presentation, a novel multi-angle FP sensor scanner designed to achieve whole body small animal imaging without sacrificing image quality will be described.

A multi-view scanner was constructed based on a planar FP sensor array that can be positioned at several angular orientations relative to the imaging target, thereby retaining the advantages of FP detection. Two reconstruction approaches were compared: iterated time reversal and adjoint-assisted optimisation. Both algorithms incorporated positivity and zero-initial-particle-velocity constraints, and exploited a 'half-time' approach to minimise the effect of acoustic heterogeneities. The possibility of improving data acquisition speed through the use of sub-sampling techniques will also be described.

The capabilities and specification of the system was carefully assessed using a range of calibration targets. The effect on the image quality with increasing the number of view angles was assessed quantitatively and qualitatively, in terms of the evenness of the spatial resolution and the level of artifacts. Finally, the ability of the system to produce high resolution whole body small animal images was demonstrated using phantoms, as well as in-vivo.

10064-133, Session PMon

Development of a tunable fiber-based MOPA laser system for photoacoustic microscopy system and design of a PDMS microfluidics device as a phantom

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Photoacoustic microscopy (PAM) is based on the detection of acoustic waves generated through the thermal expansion of tissue illuminated by a short laser pulse. Recently, fiber lasers with high repetition rates have emerged as an alternative excitation source for PAM. Here, we report a unique tunable fiber-based MOPA laser system produced specifically for PAM and also a polydimethylsiloxane (PDMS) device for phantom studies. The laser is custom developed with adjustable parameters; pulse duration (5-8 ns), pulse energy (up to 10 ?J) and repetition frequency (up to 110 kHz) and covers a broad spectral region from 500-1100 nm. The system consists of a MOPA, seeding two stages; an all-fiber supercontinuum and a harmonic generation unit in order to obtain the wavelengths of 532, 355, and 266 nm, respectively. The laser system is outstanding since the oscillator, amplifier and supercontinuum generation parts are all-fiber integrated with custom-developed electronics and software. To demonstrate the feasibility of the system, the images of several elements of USAF test target were acquired at multiple wavelengths. The lateral resolution of OR-PAM system was verified as 2.68 ?m. We also designed and developed a PDMS phantom, loaded firstly with black ink and then human blood and conducted photoacoustic experiments. PDMS devices may be used for the evaluation of PAM systems and also may serve as tissue-mimicking structures, since it is possible to make adjustments in the optical properties throughout production.

10064-134, Session PMon

Compression-tracking photoacoustic perfusion and microvascular pressure measurements

Min Choi, Roger J. Zemp, Univ. of Alberta (Canada)

Clinicians sometimes make difficult decisions about amputation based on simple fingernail compression tests where the time of re-perfusion is visualized by colorimetric changes. We aim to extend the utility of these techniques to deep tissues where colorimetric changes are not visible by eye. We do this using photoacoustic imaging. We recently demonstrated the potential of this technique for photoacoustic microvascular pressure estimation by compressing tissues to the point that blood is occluded and tracking vessels with correlated force measurements. Here we extend this method to tissue regions where no discrete vessels are necessarily visible and correlate perfusion with applied pressure and photoacoustic re-perfusion rates. Imaging is performed using a photoacoustic-micro-ultrasound realtime imaging array system. Tissue-tracking methods are used to track regions of interest over compression cycles. We demonstrate this in phantoms and in human subjects as well as transient-ischemic mouse-limb models. It is hoped that this technique will prove useful to clinicians needing to assess perfusion and microvascular function in deep tissues.

10064-135, Session PMon

Effect of small and large animal skull bone on photoacoustic signal

Qiuyun Xu, Bridget Volinski, Ali Hariri, Afreen Fatima, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States)

The brain photoacoustic imaging (PAI) can be a promising technique for the detection, diagnosis, and treatment monitoring of neurological disorders, non-invasively. The PA signal is however largely affected by the skull in both light illumination and ultrasonic detection paths. In this study, the PA signal attenuation and dispersion due to the skull bone of a mouse, a rat and a dog, ex-vivo, at the wavelengths from 450 nm to 700 nm are quantitatively explored and discussed. In addition to the study of the effect of skull on both illumination and detection paths, the effect of skull on only light illumination and that on only ultrasonic detection are also explored. This study will ultimately allow the accurate quantification required for skull modeling and aberration correction in brain PAI.

10064-136, Session PMon

Photoacoustic investigation of a neonatal skull phantom

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There is a need for continued research into the diagnosis, prevention and cure of neonatal brain disease and disorders. These disorders lead to fatalities and developmental disorders in infants. Non-invasive imaging techniques are being researched for this purpose. However, the availability of neonatal skull samples for this work is very low. A phantom can be used to simulate the neonatal skull and brain to improve imaging techniques. This study selects a phantom of polyurethane and titanium dioxide and proves its value as a replacement for neonatal skull in research. The methods used for this proof are validation of choice against the literature, transmissivity and acoustic experimentation compared to existing literature, and finally photoacoustic evaluation of the final choice to show its usefulness as a neonatal skull phantom.

10064-137, Session PMon

Modelling skull's acoustic attenuation and dispersion on photoacoustic signal

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Despite the great promising results of a recent new transcranial photoacoustic brain imaging technology, it has been shown that the presence of the skull severely affects the performance of this imaging modality. In this paper, we investigate the effect of skull on generated photoacoustic signals with a mathematical model. The developed model takes into account the frequency dependence attenuation and acoustic dispersion effects occur with the wave reflection and refraction at the skull surface. Numerical simulations based on the developed model are performed for calculating the propagation of photoacoustic waves through the skull.

10064-138, Session PMon

In vivo photoacoustic flow cytometry for sickle-cell disease staging and therapy guidance

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In vivo photoacoustic (PA) flow cytometry (PAFC) demonstrated high sensitivity for detection of many abnormal cells in the circulatory system including circulating tumor cells and malaria-infected red blood cells (RBCs). Here we demonstrate in preclinical study of PAFC's utility for detection of circulating sickle RBCs (sRBCs). Although PT and PA signal amplitudes from in vitro single sRBC in linear mode were found 2-4–fold lower than that from normal RBCs (nRBCs), we observed higher local absorption of clustered sickling-hemoglobin (HbS) in sRBCs compared to hemoglobin HbA in nRBCs. This provided spatially selective generation of nanobubbles around overheated HbS clusters only as significant PA signal amplifier. This feature then was used for in vivo PAFC selective detection and identification of rare sRBCs among high nRBCs background in mouse model of human sickle –cell disease (SCD). The differences in absorption of sRBCs and nRBCs was used for exploring a potential of fluctuation PAFC for identification of SCD. The obtained data suggest that noninvasive label-free fluctuation and nanobubble- amplified PAFC has a potential for real-time enumeration of sRBCs in vitro and in vivo, which is important for SCD staging, treatment optimization and prevention of SCD crisis by well-timed therapy including PT nanobubble-based therapy.

10064-139, Session PMon

Modified delay-and-sum reconstruction algorithm to improve tangential resolution in photoacoustic tomography

Sandeep Kumar Kalva, Manojit Pramanik, Nanyang Technological Univ. (Singapore)

In photoacoustic/optoacoustic tomography (PAT/OAT) for a circular scanning geometry, the axial/radial resolution is not variant spatially and

also do not depend on the ultrasound transducer (UST) aperture. But the tangential resolution is affected by the size of the detector aperture and is spatially variant. To counter this problem many techniques such as attaching a negative lens to the transducer surface, or using virtual detectors were proposed. However these techniques have difficulties. Therefore, a modified delay-and-sum reconstruction algorithm was proposed which can be used with the normal UST to improve the tangential resolution. In this work, we demonstrate the improvement of tangential resolution using the modified delay-and-sum reconstruction algorithm with experimental data. We have obtained more than threefold improvement of resolution in the tangential direction using non-focused and cylindrically focused USTs in a circular scanning geometry. We also observe that shape of the target object can also be preserved which is helpful for diagnosis and treatment purposes.

10064-140, Session PMon

Compact photoacoustic tomography system

Sandeep Kumar Kalva, Manojit Pramanik, Nanyang Technological Univ. (Singapore)

Photoacoustic tomography (PAT) is a non-ionizing biomedical imaging modality which finds applications in brain imaging, tumor angiogenesis, monitoring of vascularization, breast cancer imaging, monitoring of oxygen saturation levels etc. Typical PAT systems uses Q-switched Nd:YAG laser light illumination, single element large ultrasound transducer (UST) as detector. By holding the UST in horizontal plane and moving it in a circular motion around the sample in full 2ϖ radians photoacoustic data is collected and images are reconstructed. The horizontal positioning of the UST make the scanning radius large, leading to larger water tank and also increases the load on the motor that rotates the UST. To overcome this limitation, we present a compact photoacoustic tomographic (ComPAT) system. In this ComPAT system, instead of holding the UST in horizontal plane, it is held in vertical plane and the photoacoustic waves generated at the sample are detected by the UST after it is reflected at 450 by an acoustic reflector attached to the transducer body. With this we can reduce the water tank size and load on the motor, thus overall PAT system size can be reduced. Here we show that with the ComPAT system nearly similar PA images (phantom and in vivo data) can be obtained as that of the existing PAT systems using both flat and cylindrically focused transducers.

10064-141, Session PMon

Slit-enabled linear-array photoacoustic tomography with near isotropic spatial resolution in three dimension

Yuehang Wang, Depeng Wang, Ryan Hubbell, Jun Xia, Univ. at Buffalo (United States)

Slit-based photoacoustic tomography is a newly developed technique that improves the elevation numerical aperture of a linear array through acoustic diffraction. The slit, placed at the acoustic focus of a linear array, effectively forms an array of virtual detectors with high receiving angle and subsequently allows for three dimensional imaging with near-isotropic spatial resolution. However, due to the complex implementation, our original system could only image phantoms and in situ animals. The system is significantly improved in this report. In particular, we designed a slit holder that can be directly mounted to the transducer array for easy adjustment of slit width and simultaneous scanning of both the array and the slit. To enlarge the imaging field of view, we replaced the single circular optical fiber bundle with a bifurcated line fiber bundle which moved simultaneously with the array and the slit. The data acquisition system has also been updated to double the imaging speed. With these improvements, the new system can image a 4 ? 4 cm2 region within 40 seconds and the object only needs to be coupled with ultrasound gel. We successfully used the system to image phantoms and vasculatures in the palm and forearm of human volunteers.

10064-142, Session PMon

Full field-of-view photoacoustic endoscopy in vivo

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Early diagnosis and treatment of cancer are of vital importance. Novel imaging methods are in great need to achieve this. Photoacoustic endoscopy (PAE) is an emerging hybrid endoscopic technology capable of providing both functional and molecular information of intact biological tissue of internal organs in vivo. With endogenous or exogenous contrast agent, cancer formation and the corresponding vascular morphology and function changes can be detected and imaged by PAE. In addition, Utilizing multi-wavelength laser excitation, a number of important physiological parameters, such as the total hemoglobin concentration, oxygen saturation of hemoglobin, and micro-hemodynamic flux, can be obtained by PAE. Such information directly correlates with vascular angiogenesis and abnormal metabolisms and thus reflects the formation and progression of many neoplasms.

In our study, a miniaturized, simple and full field-of-view photoacoustic/ultrasonic endoscopy system was developed. A flexible coil was used to transmit the rotational torque from the rotary stage, which enables a 360o field-of-view imaging in vivo. The developed imaging catheter was fully encapsulated by a single-use protective polyamide tube. A B-scan rate up to 5 Hz (200 A-lines/B-scan) was achieved. Three-dimensional photoacoustic and ultrasound images of the rectum from a SD rat were acquired in vivo. The significantly improved imaging field-of-view, together with the flexible, simple and encapsulated catheter design, suggests that this PAE system can be of great interest for clinical translation for a variety of endoscopic applications, such as the urogenital, colorectal and gastrointestinal tract imaging.

10064-143, Session PMon

Photoacoustic microscopy with a fiber laser ultrasound detector

Liang Yizhi, Jinan Univ. (China); Lidai Wang, City Univ. of Hong Kong (China); Long Jin, Jinan Univ. (China)

The development of photoacoustic tomography (PAT) demands for novel high-performance ultrasound detectors in terms of high sensitivity, small size, broad bandwidth and inherent stability. Specifically, intravascular and related endoscopic applications require ultrasound sensors with miniature size and high sensitivity. The performance of piezoelectric detectors is limited by the tradeoff between sensor size and sensitivity. Recently, a number of photonic ultrasound sensors with outstanding performances have been demonstrated, by using high-finesse optical resonators including microring and Fabry-Perot cavities. However, the resonant sensors are susceptible to environmental disturbances and additive frequency-locking techniques are needed.

Fiber lasers have been used as light sources for the excitation of photoacoustic signals in PAT. Here we demonstrate that a compact fiber laser can also be exploited as a highly sensitive ultrasound detector. The laser contains a Bragg-grating Fabry-Perot cavity in an Er-doped optical fiber. The laser has an output with two orthogonal polarization modes which are slightly different lasing frequencies, yielding a radio-frequency beat signal. Ultrasound wave can drive harmonic vibration of the fiber and induces beat-frequency variation. The beat signal can be demodulated to reconstruct the PA signals. We demonstrate optical-resolution photoacoustic microscopy (PAM) with such a laser sensor, with an NEP of 40 Pa over a 50-MHz bandwidth, a 48-micron axial resolution and a 3.3-micron lateral resolution, respectively. The laser has a diameter of 65 microns and the compact sensor size allows for reflection-mode PAM. The detector exhibits strong resistance to environmental perturbations due to

common-mode cancellation between the two orthogonal modes, which is beneficial for practical imaging applications. The fiber-laser ultrasound detector offers a valuable tool for all-optical photoacoustic imaging techniques.

10064-144, Session PMon

Photoacoustic measurements of blood oxygen saturation in blood bags in-situ

Ruben N. Pinto, Ryerson Univ. (Canada) and St. Michael's Hospital (Canada); Karan Bagga, Ryerson Univ. (Canada) and Institute for Biomedical Engineering, Science and Technology (iBEST) (Canada); Jason P. Acker, Canadian Blood Services (Canada); Alexandre Douplik, Michael C. Kolios, Ryerson Univ. (Canada) and St. Michael's Hospital (Canada)

Blood Gas Analyzers (BGAs) are routinely used in blood banks to measure various parameters of stored blood prior to transfusion. It is hypothesized that photoacoustic (PA) technology can measure oxygen saturation (SO2), of blood within blood bags in-situ, unlike BGAs that require extraction of blood samples. This study evaluates if passive PA SO2 values are comparable to the values obtained from BGA; furthermore, the study tracks the SO2 changes every 2-7 days in blood bags over their 6-week lifespan.

PA measurements (VevoLAZR, Fujifilm VisualSonics) were carried out on 6 bags of freshly donated (NetCad, CBS) packed RBCs; PA oximetry was applied to data collected at 750/850nm, and SO2 values were obtained for 3 ROIs on each bag. Three 1ml samples were extracted per bag, using specialized syringes that minimizes external air contact (safePICO, Radiometer); syringes were then placed into a BGA (ABL800 FLEX, Radiometer).

The average correlation coefficient between PA and BGA SO2 values was 0.9910.004. PA SO2 values were 2.94% lower than BGA SO2 values; this could be attributed to the unavoidable exposure to external oxygen as RBCs are extracted from the blood bag. Variation within each PA measurement ranged from 1.00-1.87%, while BGA variation ranged between 0.20-1.15%. SO2 values were found to increase monotonically over time, with the sharpest increase (6-9%) occurring either during the third or fourth week of storage. Results demonstrate potential of PA technology to measure SO2 of stored blood in-situ. Future work will investigate links between SO2 change and biochemical or morphological changes of RBCs.

10064-145, Session PMon

Photoacoustic response of gold nanorods by using 870nm, 905nm, and 940nm high power diode lasers

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In this work, we investigate the optical absorption and the optoacoustic response of three solutions of gold nanorods that exhibit a high absorption peak at 870, 905 nm, and 940 nm, where the high power diode lasers (HPDLs) that we will use in our setup have the central wavelength of light emission, respectively. We will couple this array of HPDLs to a 7-to-1 675-?m-diameter fiber bundle though 200-μm-core optical fibers to simulate a photoacoustic endoscopy application. We will use three 870-nm and three 905-nm HPDLs, and a 940-nm HPDL to emit < 100 ns light for optoacoustic signal generation. Particular emphasis will be attributed to the design a driver circuit for 940-nm HPDL since it works with very high current (< 250 A) and optical peak power (< 500 W). We will evaluate the optoacoustic amplitude as a function of the nanoparticles concentration in distilled water until reaching a value that can ensure a good optical absorption. Then, we will calculate the corresponding absorption coefficient as a function of the optoacoustic amplitude. Also, finding the minimum concentration of gold nanorods that ensures the maximum absorption can be useful for using the minimum dose for optoacoustic imaging of human tissues. In this work we will estimate their concentration from evaluation of optoacoustic signals to simulate a real scenario of chromophore detection in optoacoustic imaging. Due to their high optical absorption in the aforementioned wavelengths, the gold nanorods can be considered good candidates as contrast agents in realistic optoacoustic applications.

10064-146, Session PMon

All optical fiber-based non-contact optical-resolution photoacoustic microscopy for practical applications

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We present a non-contact optical-resolution photoacoustic microscopy (OR-PAM) system based on optical interferometric detection. In most OR-PAM systems, microscope object lenses are used to illuminate the sample under imaging with excitation laser pulses and ultrasonic transducers to detect the photoacoustic (PA) signals generated inside the sample. However, the ultrasonic transducer should be physically contacted to the sample due to the acoustic impedance matching problem. Even worse, since the transducer is opaque in general, it blocks the beam path of the excitation beam; which makes the system bulky and difficult to align. In addition, the physical contact between the transducer and the sample can cause contamination of the sample. In this work, home-made lensed fibers are used for excitation and detection of PA signals to minimize the volume of the sample probe. The optical fibers having on-body lenses allow for easy alignment of the OR-PAM system and allows the optical detection of the generated PA signal. By utilizing a high repetition rate semiconductor laser, we can replace the generally used bulky Q-switched Nd:YAG laser and implement a low-cost practical system. The system performance was measured by imaging various arrangements of human hair as samples. Owing to the simplicity and high performance of the proposed system, it can find many practical applications in various biomedical fields.

10064-147, Session PMon

All-optical frequency domain photoacoustic microscope based on two-wave mixing in a photorefractive crystal

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The photoacoustic (PA) effect, the generation of acoustic waves due to the absorption of electromagnetic energy, forms the basis of photoacoustic microscopy (PAM). Optical Resolution Photoacoustic Microscopy (OR-PAM) provides optical absorption contrast at a micron scale, thus providing structural and functional information at the cellular level. OR-PAM typically employs nanosecond pulsed lasers as excitation sources which generates acoustic waves extending to hundreds of megahertz, depending on the length scale of the absorbers involved. Images are acquired by scanning the excitation beam, which is usually focused to a spot size of a few microns; this determines the lateral resolution. However, the use of ultrasonic transducers as detectors, constraints the axial resolution to an order of magnitude lower due to the inherently limited bandwidth of the transducer and the attenuation of higher frequencies by the acoustic coupling media. This results in highly asymmetric voxels. Here we present an All Optical Frequency Domain Photoacoustic Microscope where the photoacoustic signal is generated by an amplitude modulated CW laser and detected using a two wave mixing (interferometer) in a photorefractive crystal. This ultra-broadband optical detector offers detection bandwidths much

greater than conventional OR-PAM, e.g, 100-200 MHz. By sweeping the amplitude-modulation frequency, different length scales in the sample can be examined. The loss in efficiency of photoacoustic excitation associated with violating the stress-confinement condition when using a CW laser is overcome by using a lock-in amplifier. This method promises a high-fidelity microscopy system that does not require acoustic coupling while reducing associated costs and footprint. [supported by NIH-NEI 1R21EY023012]

10064-148, Session PMon

Enhanced photoacoustic detection of melanoma cells using gold and silver nanoparticles based on skin tissue phantom

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The various properties of gold nanoparticles (AuNPs) have been used to quickly changing the field of cancer detection and treatment. Here melanoma cells have been tagged with gold and silver nanoparticles (SNPs) to show their viability for pre-cancerous and malignant melanoma lesions. Photoacoustic(PA) detection methods have been showed an increase in sensitivity using nanoparticle (NPs), which selectively targets melanoma cancer cells.

We performed a comparative study of unmodified melanoma cells, gold nanoparticle (AuNP) tagged melanoma and silver nanoparticle (AgNP) tagged melanoma. A Q-switched, tunable Nd:YAG laser was used to irradiate cells which was located in skin tissue phantom and PA signals were measured using a ultrasonic transducer in the stationary test, the PA signal strength (PSS) was computed as the integration of the Hilbert Transform of the PA signal. The function generation and signal acquisition were performed in the PC using Labview software.

The comparison revealed that nonpigmented tumor cells do not have sufficient optical contrast for photoacoustic(PA) method whereas the AuNP and AgNP can be engineered to enhance the diagnostic power of photoacoustic(PA) imaging at a very low concentration. The results of our study have the potential to not only better develop photoacoustic (PA) detection of melanoma, but also extend the viability and use of photoacoustics into detection of otherwise unpigmented cancers especially for the early detection of cancer.

10064-149, Session PMon

Multi-modality analysis of glucose aqueous solution using photoacoustic and dielectric spectroscopy for non-invasive glucose monitoring

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The number of diabetic patients has increased greatly throughout the world. Consequently, there is a huge demand for non-invasive glucose testing to allow patients to check their glucose level without sampling blood with needles. Conventional optical spectroscopy suffers from a lack of repeatability due to high optical scattering in skin tissue. Here we present a multi-modality analysis of glucose aqueous solution using photoacoustic spectroscopy (PAS) and broadband dielectric spectroscopy (BDS). These techniques involve the direct detection of the acoustic and electromagnetic waves propagating through or reflecting from tissue without their being scattered. They therefore have potential for better tolerance to the variation of scattering. For PAS, the glucose absorption at around 1.61 um was chosen. To differentiate signals induced by the water absorption, we select another laser wavelength 1.38 um, that exhibit the same absorbance for water at 1.61 um. Furthermore, one of the two photoacoustic signals is used to normalize the variations of acoustic properties in differential signal. Measured results for glucose solutions (0-2 g/dL) showed that the differential signal has a sensitivity of 1.61%/g·dL, and a detection limit of 120 mg/dL. We also tested glucose detection with BDS (500 MHz to 50 GHz) by detecting glucose hydration bonding at around 20 GHz. Using a partial least square analysis and first derivation on broadband spectra, we obtained an RMS error 19 mg/dL and a detection limit of 59 mg/dL. Using both the low-scattering ultrasonic and microwave detection techniques, we successfully capture the glucose footprint in physiological range.

10064-150, Session PMon

All-optical photoacoustic elasticity measurements in bovine eye ex vivo

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Measuring the elasticity of the eye in vivo is valuable for the diagnosis and monitoring of eye-related diseases. In particular, liquefaction of the vitreous humour occurs with age and certain pathologies, such as posterior vitreous detachment. Elasticity can be determined by imaging shear waves, but often requires mechanical loading, which is unsuitable for clinical applications.

We propose an all-optical photoacoustic technique for measuring the elasticity of the vitreous humour. A nano-second pulse of light incident upon the vitreous humour results in thermoelastic expansion and the generation of elastic waves (compressional, shear, and surface waves). The Young's modulus can be calculated directly when two of these velocities are known. We use a laser-Doppler vibrometer to detect the particle displacement perpendicular to the surface; therefore, we record the compressional and surface waves.

We have measured the elastic properties of a bovine eye ex vivo. First, optimum wavelengths for generating elastic waves on the surface of the vitreous humour in the range of 900 nm to 1410 nm have been determined. Subsequently, quantitative measurements of the Young's modulus by analysis of the compressional and surface wave velocity of the vitreous humour were performed for different excitation wavelength. Future work will combine this approach with an optical coherence elastography technique to image the shear wave directly, toward measurements of the vitreous humour in vivo.

10064-151, Session PMon

Photoacoustic remote sensing microscopy with lock-in amplification

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Photoacoustic remote sensing (PARS) is a newly developed all-optical non-contact photoacoustic microscopy imaging technique, which is able to achieve high optical lateral resolution and high optical contrast. A low-coherence probe beam interrogates refractive index modulations at subsurface interfaces with both absorption and refractive index contrast. Recently, we have reported using lock-in amplification combing with a high pulse repetition rate excitation laser for photoacoustic imaging to reach high detection sensitivity as low as nV even in noise orders of magnitude higher than the signal. The frequency domain measures presented higher signal noise ratio (SNR) compared to the broad band time-domain measures. Here we propose to integrate lock-in amplification with PARS to obtain higher SNR signals, hence enable high resolution, high contrast all optical

non-contact photoacoustic imaging at depth beyond optical scattering limitation.

A 532nm pulsed laser with tunable repetition rate (20kHz to 600kHz) was used in our experiment as excitation laser source. A 1310 CW laser was used as probe beam, and was detected by a broad band detector connected to a lock-in amplifier. In our phantom studies with carbon fibers, 35 dB SNR was acquired by using lock-in amplification compared with only 22 dB SNR by using a broad band detector and with the same low pulse energy setting. Phantom studies on imaging depth also demonstrated PARS using lock-in amplification was able to achieve similar imaging resolution at ~5 times deeper imaging depth compared to PARS using traditional broadband time-domain measures. Therefore, the high sensitive lock-in amplification technique may be potentially greatly extend the depth of photoacoustic imaging.

10064-152, Session PMon

Photoacoustic imaging system and needles for needle tip visualization

Kaku Irisawa, Dai Murakoshi, Atsushi Hashimoto, FUJIFILM Corp. (Japan)

Visualizing the tip of medical devices like needles or catheters is a continuing topic from early 1980's. In this study, needle tip visualization system utilizing photoacoustic (PA) effect is proposed. In order to visualize the needle tip, an optical fiber was inserted into the 22 G-diameter 10 cm-length needle. The optical fiber tip is arranged on the needle bevel and fixed with black glue. The pulsed light from laser diode was transferred to the optical fiber and converted to the ultrasound in the black glue due to laser light absorption and subsequent PA effect. The ultrasound from the needle tip is detected by transducer array and reconstructed into PA images in the ultrasound imaging unit which is software-modified FUJIFILM's clinical ultrasound system. B-mode images can also be acquired and superimposed onto the PA images in this system.

For evaluating above PA imaging system and needle, the needle was punctured into a pig meat, which was observed with the PA imaging system and usual clinical ultrasound transducers. The needle tip is visualized enough at 7 and 9 cm depths with linear and convex probe, respectively, even with the steep needle puncture angle around 80 degs. Laser and acoustic outputs, and temperature increases at the needle tip were measured and well below the limits of the safety standards. This photoacoustic needle tip visualization system and needles has promising feature for clinical procedures such as real-time ultrasound-guided nerve blocking due to the easy detection of needle tip by superimposed ultrasound and PA images.

10064-153, Session PMon

Photoacoustic microscopy of oxygen metabolism at the microscopic level

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Abnormal oxygen metabolism appears in a wide spectrum of complex diseases, including cancer and ischemic stroke. High-resolution imaging of the spatially heterogeneous metabolic rate of oxygen (MRO2) in these devastating diseases may lead to improved understanding of the pathophysiology and new therapeutic strategies. However, existing imaging techniques are yet able to quantify MRO2 at the microscopic level.

To fill this gap, we have developed multi-parametric photoacoustic microscopy (PAM) for quantitative mapping of microscopic MRO2. Capitalizing on the optical absorption of endogenous hemoglobin, the primary carrier of oxygen, our multi-parametric PAM enables simultaneous quantification of the three parameters prerequisite for MRO2 calculation, including the concentration of hemoglobin (CHb), oxygen saturation of hemoglobin (sO2), and blood flow (BF). Moreover, we have developed a set of algorithms to extend these microvascular measurements to the tissue level. Briefly, we divide the PAM-imaged microvessels into micro-segments using a self-developed semiautomatic segmentation algorithm, and then interpolate the tissue-level CHb, sO2, and BF by superposing the corresponding values of individual micro-segments with model-based weight factors. Upon interpolation, we can derive the microscopic MRO2 using the Fick's law.

We have applied this enabling technology to study the metabolic heterogeneity in both ischemic stroke in the mouse brain and tumor xenograft in the mouse ear. The cerebral MRO2 map acquired by in-vivo PAM shows a striking spatial correlation with the infarct determined by terminal triphenyltetrazolium chloride staining. A spatially confined elevation in the MRO2 is observed within the ear tumor at the early stage.

10064-154, Session PMon

Multi-wavelength photoacoustic imaging for monitoring lesion formation during high-intensity focused ultrasound therapy

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Photoacoustic imaging (PAI) can be used to monitor lesion formation during high-intensity focused ultrasound (HIFU) therapy because HIFU changes the optical absorption spectrum (OAS) of the tissue. However, in traditional PAI, the change could be too subtle to be observed either because the OAS does not change very significantly at the imaging wavelength or due to low signal-to-noise ratio in general. We propose a machine-learning-based method for lesion monitoring with multi-wavelength PAI (MWPAI), where PAI is repeated at a sequence of wavelengths and a stack of multi-wavelength photoacoustic (MWPA) images is acquired. Each pixel is represented by a vector and each element in the vector reflects the optical absorption at the corresponding wavelength. Based on the MWPA images, a classifier is trained to classify pixels into two categories: ablated and non-ablated. In our experiment, we create a lesion on a block of bovine tissue with a HIFU transducer, followed by MWPAI in the 690 nm to 950 nm wavelength range, with a step size of 10 nm. In the MWPA images, some of the ablated and non-ablated pixels are cropped and fed to a neural network (NN) as training examples. The NN is then applied to several groups of MWPA images and our preliminary results show that the lesions can be identified clearly. To apply MWPAI in/near real-time, systematic feature selection is performed and the number of wavelengths is decreased from 27 to 5 while retaining adequate performance. With a fast-switching tunable laser, the method can be implemented in/near real-time.

10064-155, Session PMon

Optoacoustic imaging utilizing diode lasers in frequency domain

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Laser diodes are available at various wavelength and optical power. For optoacoustic imaging however, laser with high immediate power and short pulses are required. Laser diodes have difficulties meeting these conditions. However, when solving the optoacoustic wave equation in the frequency domain, the physical phenomena can be described using the Helmholtz equation. This allows optoacoustic imaging through measuring the frequency response of the optoacoustic system for a given number of frequencies. This can easily be achieved by amplitude modulated laser diodes.

We have developed current drivers to modulate the intensity of laser diodes

at frequencies in the Mhz region. If illuminating a sample, this gives rise to an optoacoustic signal at the corresponding frequency. Since the acoustic frequency is known and well defined, highly effective filtering methods in software and hardware can be used to discriminate noise and achieve suitable signal-to-noise ratios.

By measuring the frequency response at several frequencies, an optoacoustic image of the sample can be created. In addition, the light of different laser diodes can be modulated to slightly different frequencies. This enables the user to acquire multi-spectral data without the need for time sharing as in time domain system. Together with the wide availability of laser diodes, this will allow for optoacoustics, multi-spectral systems at low cost and high speed.

10064-156, Session PMon

Laser-generated focused ultrasound for microscale treatment on small animal brain

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Laser-generated focused ultrasound (LGFU) is a suitable modality for non-invasive, non-thermal, and micro-ultrasonic treatment of the tissue. A new LGFU system, recently developed with long-range focal depth (up to 36 mm) together with a tight focal spot (~100 um), is utilized to treat a mouse brain. Here, we first characterized LGFU performance with a tissue-mimicking gel and then with the tissue of mouse brain ex vivo. The result shows that we could generate a sufficiently high pressure amplitude for acoustic cavitation which can be used for micro-histotripsy. Such high pressure implies that our high-frequency focused ultrasound (~15 MHz) can reach down to several mm in depth, still with MPa amplitudes in peak. Then, we performed with a mouse brain and confirmed similar results for LGFU. Based on these successful results, we demonstrate that our system can be used to induce micro-ultrasonic disturbance on the mouse brain in vivo which has a transparent polymer window on top of the cortical tissue after removing a part of skull.

10064-157, Session PMon

Optimization of high-speed stimulated Raman scattering source from 545 nm to 695 nm for spectroscopic photoacoustic microscopy

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Photoacoustic microscopy (PAM) has been studied to achieve the high resolution and high contrast image by using an optical absorption property of tissue. Spectroscopic PA imaging has been typically performed by the tunable dye lasers and optical parametric oscillator (OPO). Although these lasers have a wide tuning range and high pulse energy, it is difficult to increase a pulse repetition rate of pulse laser so that an acquisition speed of PA signal is limited. On the other hand, supercontinuum source for PAM has been also reported but the wavelength selection with bandpass filters causes the low energy per band. In order to solve these slow speed and low pulse energy, the fiber laser using stimulated Raman Scattering (SRS) effect has been steadily reported as an alternative source for PAM.

In this study, for high-speed spectroscopic photoacoustic microscopy with a 300 kHz of repetition rate, we optimize the stimulated Raman scattering source from 545 nm to 695 nm corresponding to the 1st ~ 10th Stokes order of Raman shift. By tuning various parameters affecting the threshold power for each Stokes generation, we success to obtain the sufficient pulse energy (100 ~ 300 nJ) at the all ten-wavelength bands (545, 558, 572, 587, 603, 619, 636nm, 655, 674, 695 nm) and also demonstrate the high-speed spectroscopic PAM images of nanoparticles with a 300 kHz of repetition rate.

From these optimized wavelength bands obtained by the experimental study of SRS Stokes order control, we can effectively performed the spectroscopic PAM studies up to nearly 700 nm in visible region. For the samples of PAM image, we use various kinds of nanoparticles with an absorbance spectrum of 600, 650, 700 nm, respectively. Through the comparison with the UV-VIS spectroscopy results, we demonstrate that our SRS source can be a useful light source for high-speed spectroscopic PAM over 300 kHz of repetition rate.

10064-158, Session PMon

Low-cost high-power light emitting diodes for photoacoustic imaging

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We present a photoacoustic imaging system based on a low-cost high-power miniature light emitting diode (LED), which has the capability of in vivo mapping vasculature networks in biological tissue. Phantoms were used to demonstrate the feasibility of the system, while in vivo imaging the vasculature of mouse ear shows that LED-based photoacoustic imaging (LED-PAI) could have great potential for label-free biomedical imaging applications, overcoming the practical limitations of the use of bulky and expensive pulsed lasers.

10064-159, Session PMon

Characterization of multi wavelength opto-acoustic system based on high power diode lasers

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Opto-acoustic effect refers to the generation of the acoustic waves due to absorption of electromagnetic radiation. If the incident light is in visible or NIR range, then the effect is called as Opto-acoustics (OA). Initially the incident laser energy is absorbed by the tissue resulting in the generation of ultrasound, which is then detected by ultrasound detector. Compared to other optical techniques OA offers the advantages by combining the ultrasound resolution with high contrast optical imaging. In addition, it has great potential for in vivo biomedical applications because it has deep imaging ability with strong optical absorption based contrast.

Typically Nd: YAG laser is used for generation of opto-acoustic signals. However, there are some limitations with this type of laser; they are very expensive, bulky and they have low repetition rate. An alternative would be to use diode laser as an excitation source; these devices are compact, relatively inexpensive, and available in a wide variety of NIR wavelengths. However laser diode also provide much higher repetition rate (~ kHz) compared to Q-switched laser systems (tens of Hz) which allows signal to be acquired very fast. The main difficulty to overcome when using laser diodes for pulsed opto-acoustic excitation is their low peak power compared to Q-switched lasers.

Fiber optics offers advantage in terms delivery of light to the remote location with minimum loss. In this work, we present the design of two-wavelength diode lasers system operating at 870 and 905 nm. We couple light emitted by these lasers using collimator and focusing lenses. The output from 6 lasers, three of which are operating at 870 and other three are operating at 905 nm is combined separately using side by side beam combining technique with 7 to 1 fiber bundle of 200 micron core input diameter and having an output diameter of 675 micron. By combining output of several high power diode lasers in such fashion, we can achieve high peak power and sufficient energy level to get OA signal. We characterized the system by measuring the opto-acoustic signal generated from tissue mimicking phantom. The output of fiber bundle can be coupled into 600 micron endoscopic probe with high efficiency.

10064-160, Session PMon

Optoacoustic system based on high energy short pulse diode laser stacks

Sergio Rodríguez Hervas, Miguel Sánchez Rodas, Luca Leggio, Sandeep Babu Gawali, Daniel C. Gallego, Horacio Lamela, Univ. Carlos III de Madrid (Spain)

In the last few decades, high power diode lasers (HPDLs) have been introduced as alternative laser sources for optoacoustic imaging (OAI), due to their high repetition rates (a few kHz) for fast OA image acquisition, lower cost and size if compared to solid state lasers. Nevertheless, their drawbacks consist in a low energy per pulse (μJ) and a relatively highly divergent beam that needs collimation optics. At this purpose, the employment of diode laser stacks significantly increases the energy per pulse up to several mJ. The diode laser stacks imply a big challenge if compared to single emitters for several reasons. Firstly, they need very demanding electronic requirements, as forward voltages and currents of several tens of volts and hundreds of amperes, respectively. Secondly, their highly divergent beam profile requires precise collimation by means of fast axis and slow axis collimation.

In this work, we show an 808-nm diode laser stack driven with 17 V and ~ 200 A by a low-cost current driver for emitting pulses of 1 mJ at 1 kHz. Particular emphasis will be attributed to the design of the high current pulses driver and the optics employed to collimate and after focus the beam in a spot. The light spot will be applied to an ink inclusion hosted in turbid phantom. We demonstrate that our system is able to generate appreciable OA signals in turbid phantoms. This aspect represents a novelty in OAI systems because it is demonstrated that HPDLs sources can efficiently replace solid-state lasers.

10064-161, Session PMon

Multimodal optical coherence tomography and multispectral photoacoustic microscopy with a single supercontinuum source: studying the influence of the source noise

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Recent research has shown interest in multimodal applications. A novel supercontinuum source with 2 ns pulse duration and 25 kHz pulse has demonstrated its usefulness in a combined photoacoustic microscopy (PAM) and optical coherence tomography (OCT) system. This source has been further improved and combined with a suitable filter for simultaneous PAM in the visible and OCT at 1300 nm, both bands are delivered by a fiber. Wavelengths from 530 nm to 840 nm present about 40 nJ pulse energy within a bandwidth of 20 nm, while the bandwidth at 1300 nm can be broader than 400 nm with more than 30 mW mean power within a 100 nm bandwidth. These parameters make the supercontinuum source suitable for ex-vivo and in-vivo simultaneous PAM-OCT imaging.

A study was conducted to evaluate how the noise of the seed driving the supercontinuum limits the application of the supercontinuum source. The study evaluates the noise parameters within a wavelength range covering visible and IR, from 500 nm to 1600 nm. Specifically, we looked at the noise of the photoacoustic detection when using excitation beams in the visible for various bandwidths and, at the noise in OCT at 1300 nm for different pulse repetition rates. Such a study is relevant to the quest of customising furthermore this source for multimodality imaging.

10064-162, Session PMon

Photoacoustic effect from moving optical sources

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Although the photoacoustic effect is usually generated by pulsed or amplitude modulated optical beams, it is clear from examination of the wave equation for pressure that motion of an optical source in space will result in the production of sound as well. Here, the properties of the photoacoustic wave generated by moving sources in one, two and three dimensions are investigated via various analytical methods. The cases of different moving patterns, e.g. acceleration, oscillation and rotation, are considered, and acoustic waves with distinct waveforms are determined. In the regime of linear acoustics, the salient feature of a source moving in one dimension is that the acoustic amplitude increases linearly in time without bound when the optical source moves at the sound speed. The enhancement in amplitude for the wave generated by an oscillatory optical source when the peak speed reaches the sound speed is also seen to produce a series spiked waves. It is thus possible to generate periodic waves whose character ranges from sinusoidal to what appears as a series of delta functions by changing the amplitude of motion of the optical beam or its peak speed.

10064-163, Session PMon

Hand-held optical-resolution photoacoustic microscopy

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The American Cancer Society recommends regular examinations of skin lesions as the best way to find skin cancers early. Therefore a non-invasive device that can easily scan the body would facilitate such routine examinations. Optical-resolution photoacoustic microscopy (OR-PAM) offers label-free in vivo imaging with high spatial resolution by acoustically detecting optical absorption contrasts via the photoacoustic effect. We developed a compact handheld OR-PAM probe for fast photoacoustic imaging. Different from bench-top microscopes, the handheld probe provides flexibility in imaging various anatomical sites. Resembling a cup in size, the probe uses a two-axis water-immersible microelectromechanical system (MEMS) mirror to scan both the illuminating optical beam and resultant acoustic beam, yielding a 3D imaging rate of 2 Hz over a 2.5 ? 2.0 ? 0.5 mm3 volume.

In the OR-PAM probe, the optical and acoustic beams are confocally configured to maximize the signal-to-noise ratio. The lateral resolution was measured as 5.0 ?m, and the axial resolution was estimated to be 26 ?m.

The system performance was tested in vivo by imaging the capillary bed in a mouse ear. We continuously monitored the changes in the signal from the mouse ear vessels after a tail vein injection of 0.6 mL of 0.9 % saline. To demonstrate the flexibility of the handheld probe in clinical applications, we clearly imaged a red mole on a healthy volunteer's leg and imaged blood vessels under the cuticle.

10064-164, Session PMon

Photoacoustic microscopy system with PZT scanner

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Photoacoustic Microscopy (PAM) is the fastest developing biomedical imaging technology that is capable of producing high spatial resolution two-dimensional or three-dimensional images of the target biological tissues for diagnose. Two-dimensional point-by-point scanning with spherically focused ultrasound detector is commonly applied in PAM system. However, the clinical applications of the PAM system are restricted due to its limited speed and high cost. In this paper, a piezoelectric (PZT) based raster scanning system with low-cost, high-speed, deep imaging and wide field-of-view (FOV) is introduced in PAM. In this system, a commercial multimode optical fiber (CMF) is mounted on a pair of piezo bimorphs such that the natural axis of one piezo bimorph (vertical) is simply mounted perpendicular to the center plane of the other piezo (horizontal) bimorph. The vertical piezo bimorph is set to be its resonant frequency while adjusting the non-resonant horizontal piezo bimorph to its best fiber tip up deflection. For the best of stabilization and protection of the fragile components, a flexible and compact housing is fabricated for the system. This system is adjustable with high precision due to our efficient algorithm for the CMF deflection detection that will be presented in this paper. We achieved a high speed scanning mechanism with FOV of 500 um * 800 um. A pencil lead and a chicken breast are 3D imaged to demonstrate the feasibility of this PAM system.

10064-165, Session PMon

PA signal produced by PDMS phantoms with different concentrations of cells using a novel fiber laser

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A polydimethylsiloxane (PDMS) device modeled by standard soft lithograph has been used for evaluation of the performance of photoacoustic system, testing various contrast agents in order to enhance PA signal before the in-vivo experimental tests. The PDMS phantom is composed of micrometric channels having the ability to form multilevel channel features with varying sizes and depths according to biological imaging targets. It can be defined as a stable physical phantom and tissue-simulating phantom with tunable optical properties when agar based phantoms are considered. Furthermore, it has suitable optical and acoustic properties, non-toxic material related properties during the preparation and application processes. In this work, PDMS phantoms are filled with different concentrations of SKMEL-28 (malignant melanoma). We aim to reveal the evaluation of PA signal produced by these phantoms with different concentrations of SKMEL-28 exhibited the largest amount of a precursor of melanin. For this purpose, we use a tunable optical source based on a high-repetition-rate nanosecond custom fiber-laser that is a suitable for a photoacoustic microscopy system (PAM) with unique supercontinuum (600-1100 nm) all fiber laser enables for spectral separation of signals from different absorbers based on their characteristic absorption spectra and can be used for functional imaging. The PDMS phantom not only become a phantom to assess the performance of the PA system but also allows a better understanding of a characteristics of PA signal produced by different cells in vitro. This proof of concept gives insights on various biomedical and clinical applications of photoacoustic imaging via PDMS device.

10064-166, Session PMon

Multispectral photoacoustic bioimaging using low power continuous wave lasers

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We have developed a noncontact photoacoustic (PA) sensor that uses a low power intensity modulated continuous wave (CW) laser. It can be easily integrated within existing commercial optical microscopes to enable PA imaging of solids and liquids using air as the coupling medium. The PA sensor has two compartments, one is the sample chamber and the other one is a resonator column to amplify the weakly generated PA signal. The PA signal is amplified by means of standing modes in a Helmholtz Resonator.

We have developed a multispectral PA system using three wavelengths: 473 nm (Red), 533 nm (blue) and 632.8 (green) nm, each operating at 3-4 mW. The system was used to study (1) methemoglobin and (2) hemoglobin. Methemoglobin is hemoglobin in which the iron in the heme group is in the Fe3+ (ferric) state and therefore cannot bind oxygen. The person with abnormal amount of methemoglobin suffers from disorder called methemoglobinemia. A high level of methemoglobin concentration would also be observed in bleeding. Methemoglobin exhibits relatively high optical absorption at 470 nm and 630 nm compared to normal hemoglobin which has peak absorptions at 420 nm and 570 nm. At the isosbestic point, 530 nm both exhibited almost identical optical absorption. Analyses of blood at single wavelength would not exactly delineate these functional properties. Multispectral studies need to study these functional parameters. Methemoglobin was prepared by treating normal hemoglobin with sodium nitrate (NANO3). The PA studies showed that methemoglobin exhibited relatively high PA signal at 473 nm and 633 nm compared to hemoglobin, and almost same at 533 nm, as expected. The system developed can be used to study the functional properties of these two forms of hemoglobin among other applications.

10064-167, Session PMon

Arteriovenous shunts in early stage tumors using photoacoustic microscopy

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We report photoacoustic microscopy (PAM) of arteriovenous (AV) shunts and blood diffusion in early stage tumors in vivo. Studying AV shunts in tumors is critical for understanding their development mechanism and metabolic basis. However, current imaging modalities cannot provide the high spatial resolution required to detect AV shunts, nor can they measure the hemoglobin landscape of AV shunts during tumor development.

Here, using a high-resolution photoacoustic microscope, we report a

new blood oxygenation (sO2)-based disease marker induced by the AV shunt effect in tumor angiogenesis. We discovered a striking biological phenomenon: There can be two dramatically different sO2 values in bloodstreams flowing side-by-side in a single vessel. By tracing abnormal sO2 values in the blood vessels, we can identify a tumor region at an early stage. We expect that this new discovery will find many applications, such as tracing sO2-based biomarkers in internal organs and the brain in humans.

10064-169, Session PMon

Photo-induced ultrasound microscopy for photo-acoustic imaging of non-absorbing specimens

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Photo-Acoustic Microscopy (PAM) has raised a special interest in pre-clinical imaging of different animal models due to its ability to preserve the near-diffraction limited spatial resolution of optical microscopes, whilst extending the penetration depth to the mm-range. Another advantage of PAM is that it is a label-free technique -- any substance that absorbs light at the excitation wavelength can be viewed with PAM. However, not all samples absorb light sufficiently to provide contrast for imaging. While exogenous contrast agents can partially solve this problem, their use is not always feasible. This work describes a novel imaging method that makes it possible to visualize optically semi-transparent samples that lack intrinsic photoacoustic contrast, without the addition of contrast agents. A thin, strongly light absorbing layer is placed to the immediate proximity of the sample, on the excitation light path. The absorption of the excitation light to this layer generates a strong ultrasound signal that can be used to obtain a regular ultrasound image of the sample, by raster scanning point-by-point, similar to regular PAM. Since there is no need to generate the ultrasound waves, such image can be obtained on any PAM system, without any modification to the microscope. Our method was used to take full body images of drosophila larvae, which are optically nearly transparent, and do not provide sufficient contrast for direct photoacoustic imaging. Our laser-scanning PAM system consists of a 532nm pulsed laser excitation source and a 50MHz ultrasound transducer, that was used in transmission mode.

10064-170, Session PMon

Combined optical and acoustic resolution photoacoustic microscopy

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Photoacoustic Microscopy (PAM) is an emerging hybrid in vivo imaging modality combining optics and ultrasound which can provide penetration beyond the optical diffusion limit while maintaining high resolution. In Acoustic resolution Photoacoustic Microscopy (AR-PAM) deep tissue imaging can be achieved with weak optical and tight acoustic focusing. In Optical Resolution Photoacoustic Microscopy (OR-PAM), the lateral resolution can be improved by tight optical focussing. OR-PAM can clearly resolve single capillaries or even a single cell. However, the penetration depth is rather limited due to light focusing. Therefore, in summary AR-PAM can image deeper, but with poorer resolution and OR-PAM can image with very high resolution but limited imaging depth. Not many efforts have been taken to integrate both these systems together. Here, we report an integrated OR and AR PAM (OR-AR-PAM) imaging system capable of both high resolution imaging as well as low resolution deep tissue imaging on the same sample using same laser for both the system. Optical Resolution PAM (~In vivo imaging at tissue depths up to 1 mm, Lateral resolution down to 5 μm, Axial resolution down to 15 μm) and Acoustic Resolution PAM (In vivo imaging at tissue depths up to 3 mm , Lateral resolution down to 45 μm, Axial resolution down to 15 μm) is been demonstrated in a single system.

10064-171, Session PMon

High SNR optical-resolution photoacoustic microscopy using a galvanometer

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Optical-resolution photoacoustic microscopy (OR-PAM) is regarded as a promising microscopic imaging technique with optical sensitivity and high ultrasound resolution for visualizing anatomical information, functional information, and metabolic contrast. Especially, fast imaging speed is a critical issue for clinical applications. However, conventional OR-PAM systems are relatively slow because of the uses of the motorized scanning stages. Several technical approaches such as voice-coil and waterproof microelectromechnical system (MEMS) scanner are integrated in OR-PAM to improve imaging speed, however, they are still bulky and complex. In this research, we develop a small and fast OR-PAM system which is equipped with a popular galvanometer scanner in non-conducting liquid. An opto-ultrasound combiner of the OR-PAM system maintains high signal-to-noise ratio (SNR). The commercially available non-conducting liquid provides simultaneous acoustic and optical scanning of the galvanometer. Using pulsed laser repetition rate of 50 kHz, obtained B-scan speed is 125 Hz. It takes only 0.8 seconds to acquire a PA maximum amplitude projection (MAP) image with 200 ? 200 pixels along X and Y axes, respectively. The measured lateral and axial resolutions are 6.0 and 37.7 μm, respectively. Finally, volumetric PA images of microvasculatures in a mouse ear are successfully demonstrated. We believe that this new system will contribute to wide-spread of OR-PAM in various preclinical and clinical applications.

10064-172, Session PMon

Low-cost laser scanning photoacoustic microscopy system with a pulsed laser diode excitation source

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Reducing the cost and size of photoacoustic microscopy (PAM) systems can increase their clinical applicability. Pulsed laser diodes (PLDs) have recently gained attention as low-cost and small substitutes for expensive and bulky solid state lasers for PAM excitation. However, due to the limitations in PLD power and the challenges in low-loss collimation of their beam, previously reported laser diode PAM systems have utilized motor scanning with at least 128 averaging at each pixel, resulting in a relatively long image acquisition time. Here, a laser scanning laser diode optical resolution PAM system is reported. A 905 nm PLD with a maximum output peak power of 650 W is used as the excitation source. An aspheric lens and three cylindrical lenses provide the long-reaching and low-loss collimation needed for laser scanning. Two galvanometer scanning mirrors that are synchronized with the laser diode scan the beam in a focusing aspheric lens. The maximum field of view is approximately 4.7 mm ? 3.6 mm. The lateral resolution is approximately 110 μm, measured via edge spread function estimation. Using a pulse repetition rate of 1 KHz and no averaging, a 500 ? 200 pixel image is acquired in approximately four and a half minutes. PAM images of human hairs, polyethylene tubes filled with mouse blood, ex vivo mouse ear, and ex vivo porcine ovary are presented. The results indicate the feasibility of PAM imaging of vasculature in biological tissue using a low-cost and fast laser scanning laser diode PAM system leading to potential applications in ovarian tissue imaging and characterization.

10064-173, Session PMon

Photonic nanojet engineering to achieve super-resolution in photo-acoustic microscopy: a simulation study

Paul Kumar Upputuri, Mogana Sundari, Mohesh Moothanchery, Manojit Pramanik, Nanyang Technological Univ. (Singapore)

Label-free photoacoustic microscopy (PAM) with nanometric resolution is important to study cellular and sub-cellular structures, microcirculation systems, micro-vascularization, and tumor angiogenesis etc. But, the lateral resolution of a conventional microscopy is limited by optical diffraction. The photonic nanojet generated by silica microspheres can break this diffraction limit. Single silica sphere can provide narrow photonic jet, however its short length and short working distance limits its applications to surface imaging. It is possible to increase the length of the photonic nanojet and its working distance by optimizing the sphere design and its optical properties. In this work, we will present various sphere designs to achieve ultra-long and long-working distance photonic nanojets for far-field imaging. The nanojets thus generated will be used to demonstrate super-resolution photo-acoustic imaging using k-wave simulations. The study will provide new opportunities for many biomedical imaging applications that require finer resolution.

10064-174, Session PMon

Hybrid ultrasound and dual-wavelength optoacoustic biomicroscopy for functional neuroimaging

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Many neurological disorders are linked to abnormal activation or pathological alterations of the vasculature in the affected brain region. Obtaining simultaneous morphological and physiological information of neurovasculature is very challenging due to the acoustic distortions and intense light scattering by the skull. In addition, the size of cerebral vasculature in murine brains spans an extended range from several millimeters down to just a few microns, all to be recorded in 3D and over an area of several dozens of square millimeters. Numerous imaging techniques exist that excel at characterizing certain aspects of this complex network, but most only provide information on a limited spatio-temporal scale.

We present a dual-wavelength hybrid-focus optoacoustic microscope (DW-HFOAM), capable of imaging murine neurovasculature in-vivo, with high optical spatial resolution (15 ?m) over a large field of view exceeding 50 mm?. The dual wavelength imaging capability further allows for the visualization of functional blood parameters through an intact skull while pulse-echo ultrasound biomicroscopy images are captured simultaneously by the same scan head. The flexible hybrid design and high-resolution rapid imaging in 3D can be utilized for generating better insights into the architecture and function of the neurovascular system.

10064-175, Session PMon

Rapid computation of photoacoustic fields from normal and pathological red blood cells using a Green's function method

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The exact solution to photoacoustic (PA) wave equation is available for a fluid spherical source. However, this approach cannot be applied for calculating PA fields from red blood cells (RBCs) above 100 MHz because spherical approximation fails in that regime. Normal (discocyte) and some pathological (stomatocyte) RBCs can be classified as concave particles exhibiting features in PA spectra depending on particle size and direction of measurement. The PA fields from such particles can be computed by numerically solving the wave equation in 3D. Nevertheless, it is a resource intensive and time consuming task, and not well suited for rapid PA characterization of cells in microfluidics applications. Here, we apply Green's function method for calculating PA fields from nonspherical axisymmetric particles.

The Evans-Fung model was employed to generate contours of discocyte and stomatocyte states of RBCs. The theoretically constructed contours were fitted with the Legendre polynomial expansion for surface parameterization. The trapezoidal rule was used to perform numerical integration for PA field computations at two directions (along and perpendicular to the axis of symmetry).

The first minimum of PA spectrum appears at 640 MHz for discocytes and 421 MHz for stomatocytes when computed from the direction of symmetry axis. The same feature occurs at 240 and 310 MHz, respectively for those particles when measured along the perpendicular direction. The numerical results are consistent with that of PA microscopy experiments. The present approach is a simple and fast method, demonstrating that rapid characterization of cellular morphology from single-particle PA spectra is possible.

10064-52, Session 10

Label free aggressive prostate cancer identification with ultraviolet photoacoustic spectral analysis

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Prostate cancer (PCa) is the most commonly diagnosed cancer in American men for the past decades. PCa has a relatively low progression rate but the 5 year survival rate decreases dramatically once the cancer has metastasized. Differentiating aggressive from indolent PCa is critical for improving PCa patient outcomes and preventing metastasis and death. Prostate biopsy is the standard procedure for evaluating the presence and aggressiveness of PCa. The microarchitecture of the biopsied tissues visualized by histology process is evaluated by pathologists and assigned a Gleason score as a quantification of the aggressiveness. In our previous study, we have shown that photoacoustic spectral analysis (PASA) is capable of quantifying the Gleason scores of the H&E stained human prostate tissues. In this study, we attempt to assess the Gleason scores without any staining by taking advantage of the strong optical absorption of nucleic acid at ultraviolet wavelengths. PA signals were generated by wide field illumination at 266 nm and received by a hydrophone with a bandwidth of 0-20 MHz. DU145 prostate cancer cells at the concentrations of 0.8, 0.4, 0.05, 0.025 and 0.0125 million per cm3 simulating those in cancerous and

normal tissues were first attempted. The measurements were repeated for 10 times at each concentration. A correlation of 0.86 was observed between the PA signal intensities and the cell concentrations. Human PCa tissues with Gleason score 6, 7 and 8 and normal tissues were assessed. With 11 samples, a correlation of 0.89 was found between the Gleason scores and PASA slopes.

10064-53, Session 10

Improving visibility in limited-view scenarios with dynamic particle-enhanced optoacoustic tomography

Xosé Luís Deán-Ben, Lu Ding, Daniel Razansky, Helmholtz Zentrum München GmbH (Germany)

Limited-view artefacts affect most optoacoustic (photoacoustic) imaging systems due to geometrical constraints that impede achieving full tomographic coverage as well as limited light penetration into scattering and absorbing objects. Indeed, it has been theoretically established and experimentally verified that accurate optoacoustic images can only be obtained if the imaged sample is fully enclosed (>180° angular coverage) by the measuring locations. Since in many cases full angular coverage cannot be achieved, the visibility of structures along certain orientations is hampered. These effects are of particular relevance in the case of hand-held scanners with the imaged volume only accessible from one side. Herein, a new approach termed dynamic particle-enhanced optoacoustic tomography (DPOT) is suggested for accurate structural imaging in limited-view scenarios. The method is based on the non-linear combination of a sequence of tomographic reconstructions representing sparsely distributed moving particles. Good performance of the method is demonstrated in numerical simulations and in experiments consisting of dynamic visualization of the flow of suspended microspheres in three-dimensions. The method is expected to be applicable for improving accuracy of angiographic optoacoustic imaging in living organisms.

10064-54, Session 10

Hybrid single plane illumination microscopy and multispectral optoacoustic mesoscopy for imaging of postembryonic model organisms

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Single plane illumination microscopy (SPIM) is an indispensable tool in fast imaging of large organisms used for developmental biology and experimental genetics. Its high resolution and fast-acquisition enables visualization of labelled specimen prone to photobleaching. However, its imaging quality strongly decreases for opaque specimen. To overcome this limitation, a hybrid SPIM-optoacoustic mesoscopy system has been developed to complement each other. These hybrid fluorescent and acoustic readouts are increasingly important to monitor developmental processes over longer periods of time. Here we introduce the hybrid system and provide a description of this new modality and show imaging results of different organisms.

10064-55, Session 10

Thermoacoustic vascular imaging using exogenous saline contrast

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The source of endogenous contrast in Thermoacoustic imaging (TAI) is the water and ionic content in tissue. As a result, TAI can currently be used effectively for discriminating between high water content and adipose dominated tissue due to the strong endogenous differential contrast. However, discriminating between tissues with high water content is challenging because the differential contrast is quite weak. For example, visualising blood vessels against the background of high water content tissue such as muscle is challenging because the conductivity of blood (? = 2.5) is quite similar to muscle (? = 2.2). We have previously identified simple electrolytes, such as saline, as the most effective exogenous contrast agent for TAI because high conductivities can be achieved using small amount of solutes, compared to other TA contrast agents.

Building on our previous study, here we explore the use of saline in order to image vascular anatomy. Saline, as used in IV infusion, has the advantage of providing high contrast (? = 3.5) and is biocompatible, well tolerated and inexpensive. Using a cylindrical scanning TAI system based on a 3GHz magnetron, we acquired images of tissue mimicking phantoms consisting tubes filled with either blood or saline, within representative background of tissues with varying water content. This establishes the level of tissue contrast that can be discriminated effectively. Subsequently, we acquired images of ex-vivo ovine organs, with the blood vessels perfused with saline. This results in the ability to unambiguously visualise the vasculature against the anatomical landscape provided by endogenous contrast, to depths of several cm, in a way that has not been previously possible using TAI.

10064-56, Session 10

Photoacoustic triplet differential imaging for substantial background noise reduction

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In vivo photoacoustic (PA) imaging with high spatial resolution and depth penetration has remained a challenge due to the high background signal from chromophores in biological tissue. In this study, we introduce a novel imaging technique called triplet differential (TD) imaging which allows for the substantial reduction of background signals from tissue. TD imaging uses the ability of molecules to enter a triplet state via intersystem crossing from an excited singlet state. Molecules in the triplet state exhibit a spectral shift in their optical absorption spectra, creating two separate absorption peaks for each molecule's singlet and triplet states. Since the PA signal is proportional to optical absorption, a differential signal can be obtained by comparing the PA signal for molecules raised to the triplet state to those in the singlet state. We worked with methylene blue conjugated polyacrylamide nanoparticles with a polyethylene glycol dimethacrylate cross-linker (MBNP) which has a singlet peak absorption at 660nm and triplet peak absorption at 840nm. Since only certain molecules such as methylene blue can enter the triplet state efficiently, the difference in the PA signal before and after excitation of the MBNP to the triplet state is largely independent of the background noise and mainly contributed by the MBNP in the triplet state. Preliminary results have shown that up to an 8-fold increase in the PA signal of the MBNP in the triplet state can be achieved.

10064-57, Session 10

In-vitro and in-vivo high-resolution fluorescence imaging in centimeter-thick tissue via ultrasound-switchable fluorescence

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Fluorescence imaging of tumor structural, functional and molecular information has been widely investigated and is playing an important role in preclinical cancer research and clinical cancer diagnostics. It has high sensitivity and specificity with benefits of low cost, use of non-ionizing radiation and capability of multiplex imaging. However, fluorescence imaging suffers from limitations in different applications. For example, fluorescence microscopy has high spatial resolution (sub-microns) but is limited in imaging depth (<1 mm). Fluorescence diffuse optical tomography can image tissue as deep as several centimeters but is limited with poor spatial resolution (a few millimeters). We developed a high-resolution fluorescence imaging technology: ultrasound-switchable fluorescence (USF) imaging, which overcomes the limitations and achieves high-resolution fluorescence imaging in centimeter deep tissue. In USF imaging, two key components are: an excellent USF contrast agent and a sensitive imaging system. Different types of USF contrast agents have been synthesized and characterized. Several USF imaging systems have been developed. High-resolution USF imaging in centimeter-thick tissue phantoms and in-vitro tissue samples has been very successfully achieved using different contrast agents and systems. Simultaneously imaging multiple targets via multi-colored USF signals is also achieved and demonstrated. Furthermore, ex-vivo USF imaging of mouse organs and in-vivo USF imaging of mouse tumors have been studied and demonstrated. High-resolution USF images are acquired in centimeter-thick tissues via different contrast agents. The effects of biological environments on USF contrast agents are also investigated. Current challenging and future directions are summarized and discussed.

10064-58, Session 10

Development of a photoacoustic handheld probe using 2-axis MEMS scanner

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Optical resolution photoacoustic microscopy (OR-PAM) is a non-invasive, label-free, in-vivo imaging modality with microscopic resolution and high optical contrast, achieved by identifying absorption from endogenous chromophores, such as hemoglobin in the blood. OR-PAM, however, has not yet been utilized in practical medical applications because of its fixed configuration, large system size, and slow imaging speed. In this work, we developed a PA handheld probe for broader medical applications. Using MEMS technology, we reduced the size of the OR-PAM system and integrated a fast scanning function into a handheld probe. Beam guiding, ultrasound guiding, and mechanical scanning subsystems are all integrated in one handheld probe. The distal end of the probe has a 17 mm diameter scanning window. The measured lateral and axial resolutions are 10 ?m and 28 ?m, respectively. The measured B-scan and volumetric imaging speeds are 35 and 0.05 Hz each for a PA image with 700 x 700 pixels. We demonstrate in-vitro and in-vivo images of various samples, including an electro-spun microfiber, carbon fibers, and a mouse ear.

10064-59, Session 10

Multiple speckle illumination for optical-resolution photoacoustic imaging

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Optical-resolution photoacoustic microscopy offers exquisite and specific contrast to optical absorption. Conventional approaches generally involves raster scanning a focused spot over the sample. Here, we demonstrate that a full-field illumination approach with multiple speckle illumination can also provide diffraction-limited optical-resolution photoacoustic images. Two different proof-of-concepts are demonstrated with micro-structured test samples. The first approach follows the principle of correlation/ghost imaging [1, 2], and is based on cross-correlating photoacoustic signals under multiple speckle illumination with known speckle patterns measured during a calibration step. The second approach is a speckle scanning microscopy technique, which adapts the technique proposed in fluorescence microscopy by Bertolotti and al. [3]: in our work, spatially unresolved photoacoustic measurements are performed for various translations of unknown speckle patterns. A phase-retrieval algorithm is used to reconstruct the object from the knowledge of the modulus of its Fourier Transform yielded by the measurements. Because speckle patterns naturally appear in many various situations, including propagation through biological tissue or multi-mode fibers (for which focusing light is either very demanding if not impossible), speckle-illumination-based photoacoustic microscopy provides a powerful framework for the development of novel reconstruction approaches, well-suited to compressed sensing apporaches [1,2].

[1] Katz et al , Compressive ghost imaging. Applied Physics Letters, 95(13), 2009.

[2] Akhlaghi et al, Compressive correlation imaging with random illumination. Optics letters, 40(19), 2015.

[3] Bertolotti et al., Non-invasive imaging through opaque scattering layers. Nature, 491(7423), 2012.

10064-60, Session 10

Label-free high-throughput detection and quantification of circulating melanoma tumor cell clusters by linear-array-based photoacoustic tomography

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Circulating tumor cell (CTC) clusters arise from multicellular grouping in the primary tumor and elevate the metastatic potential by 23 to 50 fold compared to single CTCs. High throughout detection and quantification

of CTC clusters is critical for understanding the tumor metastasis process and improving cancer therapy. In this work, we report a linear-array-based photoacoustic tomography (LA-PAT) system capable of label-free high throughput CTC cluster detection and quantification in vivo. LA-PAT detects CTC clusters and quantifies the number of cells in them based on the contrast-to-noise ratios (CNRs) of photoacoustic signals. The feasibility of LA-PAT was first demonstrated by imaging CTC clusters ex vivo. LA-PAT detected CTC clusters in the blood-filled microtubes and computed the number of cells in the clusters. The size distribution of the CTC clusters measured by LA-PAT agreed well with that obtained by optical microscopy. We demonstrated the ability of LA-PAT to detect and quantify CTC clusters in vivo by imaging injected CTC clusters in rat tail veins. LA-PAT detected CTC clusters immediately after injection as well as when they were circulating in the rat bloodstreams. Similarly, the numbers of cells in the clusters were computed based on the CNRs of the photoacoustic signals. The data showed that larger CTC clusters have faster clearance rates, i.e., shorter lifetimes, in the bloodstreams. The results prove the potential of LA-PAT as a promising tool for both preclinical tumor metastasis studies and clinical cancer therapy evaluation.

10064-61, Session 10

Dynamics of the photoacoustic response of single-element PZT transducers to pulse burst excitation

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Achieving good signal-to-noise ratio at increased depths remains a challenge, even for photoacoustic imaging, which stimulates the search for possible contrast mechanism improvements. Both double-pulse and pulse burst excitation are shown beneficial for increasing the signal-to-noise ratio or acquiring the additional information about the sample. We propose using semiconductor laser diodes in the pulse burst regime to investigate opportunities to signal-to-noise ratio improvement based on the dynamics of the transducer response.

Photoacoustic response of the target demonstrates various effects dependent on fulfillment of the stress and / or thermal confinement conditions. However, conventional laser sources usually have low repetition rates, which leaves out the possibility to study the short-term effects without significantly rising the systems' complexity. Moreover, laser systems previously used could not offer seamless expansion of the pulse excitation sequences, due to the way the pulse pair / train is generated. Additionally, the temporal span of such systems is limited intrinsically.

In contrast, semiconductor laser diodes offer great opportunities regarding both number of pulses in the burst and inter-pulse delay times. They additionally exhibit the lowest system complexity and footprint, being rigid, robust and cost-effective.

We investigate the dynamics of pulse burst excitation responses of the single-element PZT transducers using a semiconductor laser diode based setup. We are concentrating here on the inter-pulse delay ranges of few hundred nanoseconds and low central frequency transducers as they are mainly used for clinical applications.

10064-62, Session 10

Towards early in vivo photoacoustic malaria diagnosis with 10,000-fold improvement in detection sensitivity

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Roughly 0.6 million people die each year from malaria due to lack of early diagnosis and well-timed treatment. Our previous study demonstrated great potential of in vivo photoacoustic (PA) flow cytometry (PAFC) for early diagnosis of deadly diseases with focus on cancer and thromboembolic complications. Here we demonstrate potential of advanced PAFC platforms using new laser, ultrasound transducer array and recording system to detect infected red blood cells (iRBCs) with malaria-associated pigment hemozoin which has a higher PA contrast than blood background. Mature parasites of human infecting species such as P. falciparum characteristically sequester mature iRBCs in the capillary bed and display synchrony in their reproductive cycle. To address this issue prior to clinical application, new PAFC platform was verified in a pre-clinical study using new animal models. Specifically, we used P. chabaudi (a rodent malaria species that mimics the characteristics of the most virulent human counterpart) to estimate the detection sensitivity with immature ring-stage parasites in peripheral blood, compared PA signals from the differing species, and examined the relationship between PA signal amplitudes and level of blood oxygenation. Based on previous successful trials on melanoma patients with melanin as an intrinsic PA marker, which has similar absorption as hemozoin, we believe that after additional malaria–related clinical trials, PAFC with a small 1064 nm laser and wearable a cost-effective, easy-to-use, watch-like, safe PA probe will provide malaria diagnosis in humans at parasitemia levels 10e4 -times lower than the current gold standard of diagnosis, the Giemsa-stained blood smear. It can reduce malaria-related mortality by well-timed treatment, especially in children in malaria-endemic countries.

10064-63, Session 10

Photoacoustic bio-quantification of graphene based nanomaterials at single cell level

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Arkansas Nanomedicine Center at the University of Arkansas for Medical Sciences in collaboration with other Arkansas Universities and the FDA-based National Center of Toxicological Research in Jefferson, AR is developing novel techniques for rapid quantification of graphene-based nanomaterials (GBNs) in various biological samples. All-carbon GBNs have wide range of potential applications in industry, agriculture, food processing and medicine; however, quantification of GBNs is difficult in carbon reach biological tissues. The accurate quantification of GBNs is essential for research on material toxicity and the development of GBNs-based drug delivery platforms. We have developed microscopy and cytometry platforms for detection and quantification of GBNs in single cells, tissue and blood samples using photoacoustic contrast of GBNs. We demonstrated PA quantification of individual graphene uptake by single cells. High-resolution PA microscopy provided mapping of GBN distribution within live cells to establish correlation with intracellular toxic phenomena using apoptotic and necrotic assays. This new methodology and corresponding technical platform provide the insight on possible toxicological risks of GBNs at singe cells levels. In addition, in vivo PA image flow cytometry demonstrated the capability to monitor of GBNs pharmacokinetics in mouse model and to map the resulting biodistribution of GBNs in mouse tissues. The integrated PA platform provided an unprecedented sensitivity toward GBNs and allowed to enhance conventional toxicology research by providing a direct correlation between uptake of GBNs at a single cell level and cell viability status.

10064-64, Session 10

Prussian blue nanocubes: multi-functional nanoparticles for multimodal imaging and image-guided therapy

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Imaging modalities utilize contrast agents to improve morphological visualization and to assess functional and molecular/cellular information. Here we present a new type of nanometer scale multi-functional particle that can be used for multi-modal imaging and therapeutic applications. Specifically, we synthesized monodisperse 20 nm Prussian Blue Nanocubes (PBNCs) with desired optical absorption in the near-infrared region and superparamagnetic properties. PBNCs showed excellent contrast in photoacoustic (700 nm wavelength) and MR (3T) imaging. Furthermore, photostability was assessed by exposing the PBNCs to nearly 1,000 laser pulses (5 ns pulse width) with up to 30 mJ/cm2 laser fluences. The PBNCs exhibited insignificant changes in photoacoustic signal, demonstrating enhanced robustness compared to the commonly used gold nanorods (substantial photodegradation with fluences greater than 5 mJ/cm2). Furthermore, the PBNCs exhibited superparamagnetism with a magnetic saturation of 105 emu/g, a 5x improvement over superparamagnetic iron-oxide (SPIO) nanoparticles. PBNCs exhibited enhanced T2 contrast measured using 3T clinical MRI. Because of the excellent optical absorption and magnetism, PBNCs have potential uses in other imaging modalities including optical tomography, microscopy, magneto-motive OCT/ultrasound, etc. In addition to multi-modal imaging, the PBNCs are multi-functional and, for example, can be used to enhance magnetic delivery and as therapeutic agents. Our initial studies show that stem cells can be labeled with PBNCs to perform image-guided magnetic delivery. Overall, PBNCs can act as imaging/therapeutic agents in diverse applications including cancer, cardiovascular disease, ophthalmology, and tissue engineering. Furthermore, PBNCs are based on FDA approved Prussian Blue thus potentially easing clinical translation of PBNCs.

10064-65, Session 10

Super-resolution imaging with ultrafast ultrasound and laser-activated nanodroplets

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Super-resolution ultrasound imaging techniques have shown promising potential in non-invasive imaging of deep-lying tissue. However, these methods utilize microbubbles, limiting its utility to visualization of vasculature with moving bubbles. To resolve extravascular targets, our group previously introduced a method for super-resolution ultrasound imaging based on laser-activated nanodroplets (LANDs) that repeatedly vaporize and recondense in response to optical irradiation. The method resolves the location of LANDs from the difference between two imaging frames capturing vaporization and recondensation of individual LANDs. However, since only two neighboring frames are used to produce a difference frame, this method is sensitive to noise-related errors limiting the improvement in spatial resolution. In this study, we introduce a new approach to super-resolution imaging. In our approach, ultrafast imaging, which typically captures images at over several thousand frames per second, was used for spatio-temporal compounding. Specifically, multiple successive ultrasound frames were used to obtain the difference frame with improved reliability and repeatability thus enhanced spatial resolution. To evaluate our approach, we imaged a phantom containing uniformly-distributed LANDs using an ultrasound system equipped with a linear array transducer and interfaced with pulsed laser. An ultrafast plane-wave compounding approach was used to capture ultrasound images at 6 kHz frame rate. We achieved a four-fold improvement in spatial resolution over the previous approach. In addition, three-dimensional super-resolution imaging of a phantom with microcapillaries containing LANDs was performed illustrating the robustness of our method. These results suggest that our approach has the potential for high-resolution molecular imaging of intravascular and extravascular targets.

10064-66, Session 11

Deep photoacoustic imaging with ultra-sensitive planoconcave optical microresonators

James A. Guggenheim, Edward Z. Zhang, Paul C. Beard, Univ. College London (United Kingdom)

Piezoelectric receivers are the current state of the art in ultrasound detection but these have three common disadvantages when applied to photoacoustic imaging. Firstly, high sensitivities needed for deep imaging (>1cm) require large elements leading to highly directional responses at MHz frequencies. Secondly, many detectors are fabricated from acoustically resonant materials resulting in sharply peaked frequency responses that compromise image fidelity. Finally, piezoelectric receivers are usually optically opaque so the excitation beam cannot pass through the detector. In contrast, the planar Fabry-Pérot sensor provides excellent images due to its smooth frequency response and low directional sensitivity, and is also transparent to excitation light. However, sensitivity and thus imaging depth is restricted by its relatively modest Q-factor (~4,000) which is limited by beam walk-off owing to the divergence of the tightly focused interrogation laser beam in the planar cavity. A new optical ultrasound sensing approach based on a planoconcave microresonator that addresses this limitation has been developed. This class of device comprises a planoconcave cavity with a curvature that is perfectly matched to the interrogation beam, eliminating walk-off to provide better confinement (Q-factor >100,000) and much higher sensitivity. A family of thoroughly tested sensors with -3dB bandwidths ranging from the low MHz to >40MHz demonstrate the scalability of the concept and it is shown that very low noise equivalent pressure (NEP <2mPa/√Hz) can be achieved. High-density 2D arrays of microresonator sensors fabricated using a novel approach are demonstrated featuring sensors optimised for deep-tissue (>1cm) photoacoustic imaging. These are deployed in a microresonator-based photoacoustic scanner and characterised in terms of resolution, sensitivity, field-of-view, and acquisition speed. 3D backward-mode imaging is demonstrated using realistic tissue phantoms and by visualising whole organs in mice in vivo. This novel system has the potential to provide improved image SNR and fidelity compared to conventional piezoelectric based photoacoustic scanners for the clinical assessment of deep targets such as breast tumours and malignant lymph nodes.

10064-67, Session 11

All-optical endoscopic probe for high resolution 3D photoacoustic tomography

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Photoacoustic endoscopy (PAE) has potentially broad application both as a diagnostic tool for the assessment of pathologies in hollow organ systems such as the GI tract and as a means for guiding minimally invasive surgical procedures. However, most conventional PAE probes employ mechanically scanned piezoelectric transducers at the distal end which can be technically complex and pose challenges in achieving the necessary level of miniaturisation for in vivo use. We present two novel all-optical PAE

probes, forward and side-viewing, both operating in widefield tomography mode that can overcome these limitations.

The forward-viewing probe comprises a 3 mm diameter flexible coherent fibre bundle, which consists of 50,000 fibre-optic cores, with a transparent 80 MHz Fabry-Pérot (FP) ultrasound sensor deposited at its distal end. The pulsed excitation light is coupled through all of the fibre-optic cores from the proximal end, and the generated photoacoustic waves are detected in backward mode by sequentially interrogating the FP sensor through individual fibre optic cores. The side-viewing probe is of a similar design but has a cone mirror attached at the distal end which serves two purposes. It reflects the excitation light emerging from the bundle orthogonal to its axis and directs the returning photoacoustic waves from tissue to the FP sensor. In both cases the FP sensor acts as a high density 2D array composed of 50,000 80MHz ultrasound detectors, with an element size and spacing equivalent to the diameter of the individual fibre optic cores (12 μm) and core-to-core spacing (15 μm), respectively. These characteristics allows for very fine sampling of the photoacoustic waves and thus high lateral spatial resolution and image fidelity.

The probes have been characterised in terms of their PSF, noise-equivalent pressure and field of view. The lateral field of view of the forward viewing probe is 3.5 mm in diameter. The lateral spatial resolution is 50 μm at a depth of 1 mm decreasing to 150μm at a depth of 3 mm. The axial resolution is 33 μm over this depth range. The probes have also been evaluated using a variety of tissue phantoms and ex vivo tissues and shown to provide excellent high resolution 3D images. This new approach to photoacoustic endoscopy offers significant advantages over previous distal-end piezoelectric based scanning probes. These include a high degree of miniaturisation, no moving parts at the distal end and relatively simple and inexpensive fabrication. It is anticipated that these devices will provide new opportunities for the photoacoustic assessment of cancer in the GI tract and guiding laparoscopic procedures used in abdominal surgery and foetal medicine.

10064-68, Session 11

Pure-optical photoacoustic detector based on total internal reflection

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Photoacoustic tomography (PAT) uniquely combines the optical absorption contrast and ultrasonic detection, which is superior for optical absorption detection. Owing to the extreme wide bandwidth, pure optical ultrasound detection methods have been studied, including the Mach-Zehnder interferometer, Fabry-Perot Polymer, micro-ring resonator, low-coherence interferometer. Most of them are based on optical interference mechanism. It is well-known that the acoustic waves can change medium refractive index. In this study, we proposed a new PA detection method that based on total internal reflection (TIR). The strength of the reflected light is modulated by the variation of media refractive index (RI) that caused by PA pressure waves. Unlike previous studies that directly detected the intensity of the reflected beam, we explored novel methods to sense very weak modulation signals in the laser intensity, and achieved a higher sensitivity and system stability. Both phantom and animal experimental results demonstrated this novel method can provide a simple and broadband PA imaging.

10064-69, Session 11

Sub-sampled multi-beam Fabry-Perot photoacoustic scanner for fast 3D imaging

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The planar Fabry Perot (FP) photoacoustic scanner has been shown to provide exquisite high resolution 3D images of soft tissue structures in vivo to depths up to approximately 10mm. However, a significant limitation of current embodiments is the long acquisition times (~5 minutes) they provide. This is because the FP sensor is optically addressed by sequentially scanning a single focused interrogation laser beam over its surface in order to map the incident photoacoustic waves. Significant reductions in acquisition time can be achieved by scanning multiple interrogation beams in order to parallelise the detection. Thus, by using a novel 8 beam scanner and a 200Hz excitation laser, 3D high resolution images (100 x 100 detection points) can be acquired within 10 seconds. In addition, video rate acquisition (17pfs) is achievable for 2D images (100 detection points). To further increase 3D image acquisition speed, a novel scanner architecture employing 24 interrogation beams and optimised sub-sampling has been developed. A specific challenge with multi-beam scanning relates to the fact that the FP sensor optical thickness is non uniform; the optimal bias wavelength is therefore different for each beam. In order to minimise the sensitivity variations across the 24 beams that this would produce when using a single interrogation laser, a new approach to optimally biasing the sensor has been developed. This is based on forming a map of the sensor optical thickness and identifying the optimum spatial alignment of the 24 interrogation beams via a statistical analysis of the sensitivity distribution over the scan area. By sub-sampling, a significantly fewer number of scanning events is required for constructing an 'full-size' image, e.g. for a 100point x100point image with a typical sub-sampling rate of 25%, just over 100 scanning events (10000 x 25% / 24beams) are required. Furthermore, a novel high pulse repetition rate (400Hz PRF) excitation fibre laser was used. These developments have resulted in a dramatic reduction in image acquisition time and a high resolution 3D image comparable to that of a single beam scanner can now be acquired within 2 seconds. It is anticipated that the significant increase in acquisition speed provided by this new scanner design will pave the way to fast 3D clinical imaging of dynamic physiological events with unprecedented image resolution.

10064-70, Session 11

A novel fibre laser for fast multiwavelength laser scanning OR-PAM

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Laser-Scanning-Optical-Resolution Photoacoustic Microscopy (LSOR-PAM) involves raster scanning a focused laser beam over the surface of a tissue sample and using a stationary ultrasound detector to record the generated photoacoustic signals. The advantage of LSOR-PAM over conventional OR-PAM is that it obviates the need for mechanically scanning the ultrasound detector (or the sample) thus offering the prospect for fast image acquisition. Realising this potential however requires an excitation source with a high PRF, typically of the order of several hundreds of kHz. Fibre lasers can provide these high PRFs as well as the necessary μJ pulse energies, high beam quality (M2<1.1) and nanosecond pulse durations. However, fibre lasers typically only operate at a single wavelength, which does not allow spectroscopic measurements to be made. This limitation can be overcome by exploiting non-linear effects observed in optical fibres when operating at high peak powers. Stimulated Raman scattering can provide access to a range of closely spaced (<15nm) discrete wavelengths ranging from 500 to 600nm and has previously been exploited for OR-PAM imaging; however, the PRF was limited to 40kHz for in-vivo imaging and required mechanically rotating a filter wheel to select the excitation wavelength resulting in relatively long acquisition times.

A novel tunable fibre laser with the ability to electronically switch wavelengths (e.g. 546, 560 and 574nm) on a microsecond timescale while operating at a PRF of 500kHz has been developed. This unique capability enables wavelength tuning between consecutive laser pulses thereby minimising errors due to motion of the tissue sample as well as permitting fast image acquisition: for example, 2000?2000 detection points over 5mm?5mm area can be acquired at two wavelengths in 8 seconds. The fibre laser which provides a high beam quality (M2<1.2), pulse durations of 1.3ns and μJ pulse energies was combined with an LSOR-PAM system

based on a fibre optic sensor. To demonstrate the ability of the system to image at high frame rates (10 fps) at two wavelengths, a tube phantom containing flowing microspheres of two different colours was imaged. The colour of single microspheres as well as their flow velocity was determined. The system was further used to image dynamic changes in SO2 and flow in the microvasculature of a mouse ear. This novel excitation source provides new opportunities for rapid multiwavelength imaging of the structure and function of the microvasculature at unprecedented frame rates.

10064-71, Session 11

Non-interferometric deep optical resolution photoacoustic remote sensing microscopy

Parsin H. HajiReza, Univ. of Alberta (Canada) and illumiSonics Inc. (Canada); Kevan L. Bell, Wei Shi, Univ. of Alberta (Canada); Roger J. Zemp, Univ. of Alberta (Canada) and illumiSonics Inc. (Canada)

A novel all-optical non-contact photoacoustic microscopy system is introduced. The confocal configuration is used to ensure detection of initial pressure shock wave-induced intensity reflections at the subsurface origin where pressures are largest. Phantom studies confirm signal dependence on optical absorption, index-contrast, and excitation fluence. Taking advantage of a focused1310 nm interrogation beam, the penetration depth of the system is improved to ~ 2mm for an optical resolution system. High signal-to-noise ratios (>60dB) with ~ 2.5 cm working distance from the objective lens to the sample is achieved. Real-time in-vivo imaging of microvasculature and melanoma tumors are demonstrated.

10064-72, Session 11

Laser-generated ultrasound for high-precision cutting of tissue-mimicking gels

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Laser-generated focused ultrasound has shown great promise in precisely treating cells and tissues by producing controlled micro-cavitation within the acoustic focal volume (<100 um). However, the previous demonstration used cells and tissues cultured on glass substrates. The glass substrates were found to be critical to cavitation, because ultrasound amplitude doubles due to the reflection from the substrate, thus allowing for reaching pressure amplitude to cavitation threshold. In other words, without the sound reflecting substrate, pressure amplitude may not be strong enough to create cavitation, thus limiting its application to only cultured biomaterials on the rigid substrates.

By using laser-generated focused ultrasound without relying on sound-reflecting substrates, we demonstrate free-field cavitation in water and its application to high-precision cutting of tissue-mimicking gels. In the absence of a rigid boundary, strong pressure for cavitation was enabled by recently optimized photoacoustic lens with increased focal gain (>30 MPa, negative pressure amplitude). By moving cavitation spots along pre-defined paths through a motorized stage, tissue-mimicking gels of different elastic moduli were cut into different shapes (rectangle, triangle, and circle), leaving behind the same shape of holes, whose sizes are less than 1 mm. The cut line width is estimated to be less than 50 um (corresponding to localized cavitation region), allowing for accurate cutting. This novel approach could open new possibility for in-vivo treatment of diseased tissues in a high-precision manner (i.e., high-precision invisible sonic scalpel).

10064-73, Session 11

Temporal evolution of low-coherence reflectometry signals in photoacoustic remote sensing microscopy

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We recently discovered that strong reflectivity modulations occur when a pulsed laser excites an absorption interface with an existing refractive index contrast. These modulations are observed using a low-coherence interrogation beam co-focused and co-scanned with an excitation beam to form high-resolution all-optical photoacoustic images. We call this new form of microscopy Photoacoustic Remote Sensing (PARS). To better understand the mechanism, analytical models were created of the time-evolution of these PARS signals. Shock waves propagating from the absorption interface create refractive index steps that form a time-varying multi-layer etalon. Besides an initial-pressure reflectivity change, GHz-modulations are predicted due to the propagating etalon effect. The characteristics of these modulations are related to the optical coherence length of the probe beam and the intrinsic optical properties of the sample. 1D plane-wave and 3D Mie-theory-based analytical models are compared with finite-difference time-domain simulations and experiments involving phantoms with different absorption- and refractive-index interfaces. Experimentally-observed modulations are detected with extremely high signal-to-noise ratios in phantoms and animal models. The newly predicted modulation mechanism offers a promising signature for deep all-optical absorption-contrast imaging with high fidelity.

10064-74, Session 11

All-optical optoacoustic microscopy based on pi-FBG ultrasound sensors

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Optoacoustic microscopy (OAM) and non-linear microscopy modalities have recently been combined and grant an extended combined tissue contrast. Thus, hybrid systems allow multi-modal, fast and label-free imaging of anatomical features in biological specimen at μm resolution. However, most applied sensors are based on the piezoelectric effect, where sensitivity is proportional to the detector size. This limits possible miniaturization of ultrasound detectors and complicates reflection mode designs as well as the addition of an OAM modality to an existing microscopy setup. As a consequence, transmission mode OAM is commonly limited to thin ex-vivo samples prepared on petri dish or thin in-vivo samples, e.g. mouse-ear, that have a limited representativeness. We introduce a pi-phase-shifted fiber Bragg grating (ϖ FBG) for the optical detection of ultrasound in an all-optical optoacoustic microscope. The ϖ FBG offers an ultra-small footprint while achieving a higher sensitivity over piezoelectric sensors of comparable size. This allows to locate the ϖ FBG in close proximity to a sample, regardless of the geometry of the setup, including reflection mode OAM. Additionally, we demonstrate coherence-restored pulsed interferometry (CRPI), allowing a super sensitive interrogation of the ϖ FBG and a high stability towards thermal and vibrational noise. In this article, we introduce both the CRPI interrogation and the ϖ FBG sensor and characterize the system in terms of sensitivity and bandwidth. Furthermore, we compare bright-field microscopy and OAM images of biological specimen, highlighting the advantage of complimentary contrast and underlining the performance of the ϖ FBG sensor.

10064-75, Session 12

Real-time display and functional optical-resolution photoacoustic microscopy with high-speed two wavelength illumination

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Optical-resolution photoacoustic microscopy (OR-PAM), has been widely used and studied as noninvasive and in-vivo imaging technique, can achieve a high resolution and high contrast image. OR-PAM is combined with optical absorption contrast and detection of acoustic wave generated by thermal expansion. Recently, nanoparticles and dyes have been used as contrast agents of OR-PAM. To obtain functional OR-PAM image such as a distribution image of blood vessels and nanoparticles, a tunable dye laser or optical parametric oscillator (OPO) should be needed at more two wavelength. However, because these lasers have a low pulse repetition rate (10 Hz ~ 10 kHz), a functional OR-PAM image with real-time display has been limited.

In our previous study, we demonstrated high-speed OR-PAM using an Ytterbium fiber laser and a graphics processing unit (GPU) technique at 300 kHz-pulse repetition rates. Although this Ytterbium fiber laser has a high pulse repetition rate, it is not comfortable for functional imaging owing to lasing at only single wavelength. Therefore, in this study, we used a high-speed interlaced illumination method at 532 nm and 1064 nm for real-time display functional OR-PAM. For high-speed interlaced illumination of two wavelength, we applied second harmonic generation effect and a high-speed optical switching using an electro-optic modulator. Therefore, we could obtain maximum amplitude projection (MAP) images about distributions of blood vessels and nanoparticles, simultaneously, with 500 x 500 pixels and a real-time display of approximately 0.5 fps.

10064-76, Session 12

Super-resolution atomic force photoactivated microscopy of biological samples

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Optical microscopy (OM) and photoacoustic microscopy (PAM) have previously been used to image the optical absorption of intercellular features of biological cells. However, the optical diffraction limit (~200 nm) makes it difficult for these modalities to image nanoscale inner cell structures and the distribution of internal cell components. Although super-resolution fluorescence microscopy, such as stimulated emission depletion microscopy (STED) and stochastic optical reconstruction microscopy (STORM), has successfully performed nanoscale biological imaging, these modalities require the use of exogenous fluorescence agents, which are unfavorable for biological samples. Our newly developed atomic force photoactivated microscopy (AFPM) can provide optical absorption images with nanoscale lateral resolution without any exogenous contrast agents. AFPM combines conventional atomic force microscopy (AFM) and an optical excitation system, and simultaneously provides multiple contrasts, such as the topography and magnitude of optical absorption. AFPM can detect the intrinsic optical absorption of samples with ~8 nm lateral resolution, easily overcoming the diffraction limit. Using the label-free AFPM system, we have successfully imaged the optical absorption properties of a single melanoma cell (B16F10) and a rosette leaf epidermal cell of Arabidopsis (ecotype Columbia (Col-0)) with nanoscale lateral resolution. The remarkable images show the melanosome distribution of a melanoma cell and the biological structures of a plant cell. AFPM provides superior imaging of optical absorption with a nanoscale lateral resolution, and it promises to become widely used in biological and chemical research.

10064-77, Session 12

Frequency domain optical resolution photoacoustic and fluorescence microscopy using a modulated laser diode

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We report on simultaneous frequency domain optical-resolution photoacoustic and fluorescence microscopy with sub-μm lateral resolution. Photoacoustic waves and modulated fluorescence are generated in chromophores by using a sinusoidally modulated diode laser. The excitation light is focused to the sample using a high NA objective lens. Fluorescence is collected by the same objective in a confocal configuration and detected by an avalanche photo diode. Photoacoustic waves are recorded on the opposite side of the sample using a hydrophone. Both, the photoacoustic and the fluorescence signals are simultaneously recorded using a lock-in technique. We show that photoacoustic and fluorescence images provide complementary information. This allows the discrimination between different chemical compositions of structures.

Commonly, scanning photoacoustic microscopes are realized in time-domain using short-laser pulses for excitation of the photoacoustic signals. When using short laser pulse excitation, the generated photoacoustic pressures can be orders of magnitude higher than for frequency domain excitation. However, we demonstrate that for sub-micrometer resolution imaging, frequency domain photoacoustic microscopy is able to compete with time domain measurements concerning the signal-to-noise ratio. For this, we present a formalism to compare the signal-to-noise ratios expected for both methods. We conclude that if one has to meet the maximum permissible exposure limits, similar signal-to-noise ratios can be achieved. Finally, we present images of e.g. labeled cells and blood smears to demonstrate the importance of multimodality. E.g., in certain hemoglobinopathies the red blood cells exhibit luminescence. This information could be easily missed if the blood is only inspected by photoacoustic microscopy.

10064-78, Session 12

Combined synthetic aperture focusing technique and three-dimensional deconvolution for resolution enhancement in photoacoustic microscopy

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Acoustic-resolution photoacoustic microscopy (ARPAM) plays an important role in studying the microcirculation system with deep penetration. High lateral resolution of ARPAM is achieved by using high numerical aperture (NA) acoustic transducer. The deteriorated lateral resolution in out-of-focus region can be alleviated by synthetic aperture focusing technique (SAFT). Previously, we reported a three-dimensional (3D) deconvolution ARPAM to improve both the lateral and axial resolutions in the focus region. In this study, we systematically present our extension of resolution enhancement to the out-of-focus region based on two dimensional (2D) SAFT combined with the 3D deconvolution (SAFT+Deconv). Depth-independent lateral resolutions provided by SAFT, together with inherently depth-independent axial resolution determined by transducer's bandwidth, ensure a depth-independent point spread function (PSF) for 3D deconvolution algorithm

both in focus region and out-of-focus region. We built a dark-field illumination ARPAM system with a 50 MHz focused transducer of 0.44 NA to validate our SAFT+Deconv method. Imaging of 6 ?m carbon fiber show that SAFT+Deconv ARPAM improves the -6 dB lateral resolution in an extended depth of focus (DOF) of 2 mm from 60—300 ?m to 30—35 ?m depending on the distance from the transducer focal point, -6 dB axial resolution from 30 ?m to 15 ?m. The signal-to-noise ratio is also increased. The resolution enhancement in three dimensions is also validated by in vivo imaging the dorsal subcutaneous microvasculature of a mouse. Our results suggest that SAFT+Deconv ARPAM may allow fine spatial resolution with deep penetration and extended depth of focus in biomedical applications of photoacoustic imaging.

10064-79, Session 12

Label-free nonlinear photoacoustic microscopy of biological samples using a femtosecond laser

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In linear photoacoustic (PA) microscopy (PAM), the axial resolution is acoustically defined, which can be improved by using high frequency ultrasonic transducers with wide bandwidths. However, high frequency ultrasound detection leads to compromised penetration depth. Here, by utilizing ultrashort laser pulses (pulse width 55 fs, wavelength 805 nm), we demonstrate nonlinear label-free PAM of in vitro cell culture with optically defined spatial resolutions in all dimensions. First, we built a transmission-mode non-linear PAM system based on a femtosecond laser. The imaging results on different biological samples (e.g.,mouse brain slices) have all shown a clear non-linear relationship between PA signal amplitudes and laser pulse energies. Then we imaged in vitro macrophages in a petri dish, with a low laser pulse energy of several nJ. Repeated imaging of the same group of cells showed no damage to the cells. Additionally, with depth scanning, we acquired a three-dimensional image of a single layer of cells, with an axial resolution of a few micrometers, much finer than the acoustically defined axial resolution. The non-linear PAM with optically defined 3D resolutions is useful for label-free live cell imaging. Moreover, the near-infrared wavelength can potentially offer deep penetration for various biological applications.

10064-80, Session 12

Synthetic light-needle photoacoustic microscopy for extended depth of field

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Photoacoustic microscopy (PAM) has been extensively applied in biomedical study because of its ability to visualize tissue morphology and physiology in vivo in three dimensions (3D). However, conventional PAM suffers from a rapidly decreasing resolution away from the focal plane because of the limited depth of focus of an objective lens, which deteriorates the volumetric imaging quality inevitably. Here, we propose a novel method to synthesize an ultra-long light needle to extend a microscope's depth of focus beyond its physical limitations with wavefront engineering method. Furthermore, it enables an improved lateral resolution that exceeds the diffraction limit of the objective lens. The virtual light needle can be flexibly synthesized anywhere throughout the imaging volume without mechanical scanning. Benefiting from these advantages, we developed a synthetic light needle photoacoustic microscopy (SLN-PAM) to achieve an extended depth of field (DOF), sub-diffraction and motionless volumetric imaging. The DOF of our SLN-PAM system is up to 1800 μm, more than 30-fold improvement over that gained by conventional PAM. Our system also achieves the lateral resolution of 1.8 μm (characterized at 532 nm and 0.1 NA objective), about 50% higher than the Rayleigh diffraction limit. Its superior imaging performance was demonstrated by 3D imaging of both non-biological and biological samples. This extended DOF, sub-diffraction and motionless 3D PAM will open up new opportunities for potential biomedical applications.

10064-176, Session PTue

PMUT+ASIC integrated platform for wide range ultrasonic imaging

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We propose an integrated platform of Aluminum Nitrate (AlN) based Piezoelectric Micromachined Ultrasonic Transducer (pMUT) phased array with Application Specific Integrated Circuit (ASIC) for medical imaging and industrial diagnosis. The ASIC provides wide driving range for frequencies between 100 kHz and 5 MHz and channel-scalable, programmable application adaptive transmitting beamformer. The system supports operation in various media, including gasses, liquids and biological tissue. The scan resolution for 5 MHz operation is 68 ?m in air. The beamformer covers a test volume from -30° to +30° with a step of 3° and scan depth of 10 cm. The ASIC system features low noise receiver electronics, power saving transmission circuitry, and high-voltage drive of large capacitance transducer (up to 500 pF). Integrated pMUT phased array consists of 4 channels of single-membrane ultrasonic transducer of 400 nm deflection and 20 pF feed-thru capacitance, which produce 15 Pa pressure at 500 ?m distance from the surface of the transducers. The active area of the ASIC is (700?1490) ?m2, which includes channel scalable TX, 8-channale low noise RX, digital back end with autonomous beamformer and power management unit. The system is battery powered with 3.3V-5V standard supply, representing a truly portable solution for ultrasonic applications.

Given the CMOS-compatible fabrication process for the AlN pMUTs, dense, miniaturized arrays are possible. Furthermore the smooth surface of dielectric AlN renders optical quality MEMS surfaces for integration in miniaturized photonic + ultrasound microsystems.

10064-177, Session PTue

Detection of ICG at low concentrations by photoacoustic imaging system using LED light source

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Recently, various type of photoacoustic imaging (PAI) that can visualize properties and distribution of light absorber have been researched. We developed PAI system using LED light source and evaluated characteristics of photoacoustic signal intensity versus Indocyanine Green (ICG) concentration. In this experiment, a convex type PZT array transducer (128-elements, 3.5MHz center frequency) was used to be able to transmit and receive ultrasound and also detect photoacoustic signal from the target object. The transducer was connected to the PAI system, and two sets of LED light source that had 850nm wavelength chip array were set to the both side of the target object. The transducer head was placed at a distance of 20 mm from the target in the water bath. The target object was a tube filled with ICG in it. The tubes containing ICG at concentrations from 300 nanomolar/liter to 3 millimolar/liter were made by diluting original ICG solution. We measured the photoacoustic signal strength from RF signal generated from front side of the ICG in the tube, and the results showed that the intensity of the signal was almost linear response to the concentration in log-log scale.

10064-178, Session PTue

A high-speed multi-channel photoacoustic tomography system for living animal imaging

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As an important molecular imaging modality, photoacoustic imaging combine the advantage of optical imaging and ultrasonic imaging. The photoacoustic imaging can provide high spatial-resolution images of endogenous and exogenous contrast agents distribution in tissues with non-invasive and non-ionizing. In recent years, the photoacoustic imaging technology is developed rapidly, especially in methodology and medical applications. The multiple spectrum, multiple dimension and multi-modality imaging fusion are development direction of photoacoustic imaging. Here we build up a high-speed multi-channel photoacoustic tomography imaging system to improve the spatial-resolution and imaging speed. The system consists of a laser, a transducer, an optical fiber, an animal displacement device and a data acquisition system . A phantom is placed in the center of the transducer and irradiated by infrared laser. The optical signal is converted to ultrasonic signal through the photoacoustic effect then the ultrasonic signal is measured by the data acquisition system. We developed a photoacoustic reconstruction algorithm based on model. The imaging view of this system is 20mm*20mm*120mm and the image resolution is 150?m. The imaging time is ten frames per second. Photoacoustic reconstructed images show a clear outline of the phantom. The system's effectiveness was verified in phantom experiments with injection of a contrast agent. Results confirmed that our system achieves the original goal in terms of the image quality and hardware performance. Furthermore, this system also has another advantage that it can be integrated with fluorescent molecular tomography and bioluminescence tomography.

10064-179, Session PTue

Image-guided laser treatment of neurodegenerative diseases by ablation of protein aggregates

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Abnormal protein aggregation is a key diagnostic feature of age-progressive neuropathies including Huntington's, Parkinson's, and Alzheimer's diseases, and also accompanies cardiac aging and hypertension. The specific causes of aggregate accumulation may vary, but the resulting cytoplasmic and intra-nuclear inclusions contribute to cellular dysfunction, and eventually cell death, via complex mechanisms. Transgenic C. elegans models of protein aggregation provide valuable insights into age-dependent accumulation of damaged proteins, risk factors, and mechanisms and allow to develop new therapies, including chemicals directly interacting with protein aggregates. However, to date, no intervention has yet been shown to directly remove previously-deposited aggregates, or to allow cellular defense mechanisms to remove them and thus restore normal functioning. Here we propose a novel method to disrupt protein aggregates at the level of single cells or whole organisms with direct laser-based photothermal ablation. This methodology provides a unique opportunity to study basic cell biochemistry and natural defense processes without the use of biologically active chemicals.

We have demonstrated that laser disruption protein aggregates in C.elegans model can delay the onset of paralysis and extend the lifespan of nematodes expressing a huntingtin-like transgene in their muscle. Laser-treated worms lived substantially and significantly longer than untreated controls ($p < 0.003$). Aggregate disruption by laser confers benefits that extend beyond the treated muscle cells. We also discussed a possible use of nanotechnology to enhance the specificity and safety of aggregate disruption through the use of molecular specific targeting of clusters.

Photoacoustic and photothermal microscopy were used for imaging of interaction between aggregates and nanoparticles and to optimize protein targeting.

10064-180, Session PTue

Initial ex vivo experience in characterization of plaque vulnerability using multi-wavelength photoacoustic imaging

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Multi-spectral photoacoustic (PA) imaging is currently explored to aid in the diagnosis of atherosclerosis in carotid arteries. Using multiple wavelengths, PA has the potential to reveal vital morphological information in plaques such as intraplaque hemorrhages, lipid pools, and the fibrous cap. In this study, we used multispectral PA and plane wave ultrasound (PUS) hybrid-imaging to reveal the composition of human plaques ex-vivo.

A fully-integrated, hand-held photoacoustic probe was used, consisting of four stack of diode lasers (Ep = 1 mJ, tp = 130 ns, ? = 808, 915, 940, 980 nm, QUANTEL, FR) and a linear array transducer (fc = 7.5 MHz, ESAOTE, NL). An intact endarterectomy sample, obtained at the local hospital, was immersed in water. The probe was positioned in the cross-section of the plaque and mechanically scanned in the longitudinal direction. Next, the sample was rotated by 10° steps and the measurements were repeated for 36 angles.

The resulting 3D reconstructions of PA/PUS identified two distinct absorbers in two different locations. The most distal absorber responded merely to the 808 nm laser pulse, which might indicate the presence of an intraplaque hemorrhage, whereas the proximal absorber generated sufficient PA signals for each wavelength.

In the future, the number of samples will be increased and histologic comparison will be done for each sample to identify the absorbers inside the samples.

10064-181, Session PTue

Photoacoustic signal detection via atomic force microscopy cantilevers: a theoretical and simulation study

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In this work, atomic force microscopy (AFM) cantilever is applied for detection of photoacoustic signals. A theoretical and simulation study about the dynamics of the cantilever that is driven by the photoacoustic force is presented approximating the dynamics of cantilever in water as the forced mass-spring-damper system. The results show the oscillation characteristics of the cantilever is strongly related to both laser and absorber parameters. And also it is indicated that the generated photoacoustic wave from microlevel samples leads to an oscillation amplitude in several nanometres level which is large enough to be measured in an actual AFM system.

10064-182, Session PTue

Toward anatomically realistic phantoms for photoacoustic imaging: A stable, non-toxic tissue-mimicking material with tuneable optical and ultrasonic properties

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Phantoms are crucial for the development of photoacoustic (PA) imaging systems, and they will become increasingly important for training as PA imaging is introduced into clinical practice. Ideally, phantoms for this modality comprise tissue-mimicking materials with optical and ultrasonic properties that can be precisely and independently controlled, which are stable over time, and which are non-toxic and low-cost. In this study, we demonstrated that a compound based on mineral oil satisfies these criteria, and that it can be used to create heterogeneous phantoms with vessels, nerves, and embedded resolution targets. Optical scattering was tuned with the addition of TiO2; optical absorption, with the addition of dyes. The optical properties, which were measured with a time-resolved system with wavelengths from 650 to 860 nm, spanned physiological ranges. The acoustic properties were tuned by the addition of glass spheres and paraffin wax. Ultrasound attenuation, as measured in reflection mode using a transducer centred at 5 MHz, matched that of different human tissues. Co-registered multi-wavelength PA and US images were acquired with a system that included a commercial US scanner, an optical parametric oscillator, and linear-array US imaging probes. With PA imaging, structures within the phantoms that had identical ultrasonic appearances and different optical properties could be differentiated with PA imaging. The textures of the US images were very similar to those encountered in clinical practice. We conclude that these mineral oil compounds are ideally suited to PA tomography and microscopy, and that they could be widely used in new generations of phantoms.

10064-183, Session PTue

Spatial interference encoding patterns based super resolved photoacoustic microscopy

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Single sensor (pixel) signals require scanning of the sample in order to obtain spatial information. In this paper we show that using interference, optically induced signals can be reconstructed when recorded using interference pattern excitation, rather than a point illumination. This method reduces the need for dense scanning and requires a small number of scans, or can eliminate the need for scanning in some cases. It is shown that this method can be used in particular in photo-acoustic imaging. Numerical as well as preliminary experimental demonstration presents the feasibility of the new concept for super resolved photo-acoustic imaging.

10064-184, Session PTue

Ultrasound modulation of bioluminescence generated inside a turbid medium

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Bioluminescence optical imaging (BLI) is a useful non-invasive technique for small animal imaging to access the pathological information of biological tissue in vivo. However, due to strong optical scattering of tissue, a major drawback is the loss of spatial resolution and quantitative accuracy to the underlying unknown and significant optical signal attenuation. This research demonstrates a novel hybrid 'acousto-optic' imaging platform that images low light level bioluminescent sources modulated at US frequency inside an optically scattering medium. This produces an US modulated light 'beacon' within the tissue in the region of US focus that reduces the effects of light scattering and improves the spatial resolution. The system consists of a continuously excited 3.5 MHz US transducer applied from the top of a tissue like 'phantom' of known optical properties (absorption and scattering coefficient) embedded with bio - or chemiluminescent sources that are used to mimic small animal experiments. Scanning US over the turbid medium modulates the luminescent sources deep inside tissue at several US scan points. These modulated signals are recorded by a photomultiplier tube that uses lock-in amplifier to generate a 1D profile. Spatial resolution is dependent on US frequency. Indeed, high frequencies enable a small focal volume, leading to a better resolution but a lower SNR. First experimental results show that US enables to localise small luminescent sources (around 2mm wide) deep (~20mm) inside a tissue phantom having a scattering coefficient of 60cm-1. Two sources separated by 10mm could be resolved 20mm inside a chicken breast.

10064-185, Session PTue

Multimodal optoacoustic and multiphoton microscopy of human carotid atheroma

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Carotid artery atherosclerosis is a main cause of stroke. Understanding atherosclerosis biology is critical in the development of prevention and treatment strategies. Consequently, there is a demand for advanced tools investigating atheroma pathology. We present hybrid optoacoustic and non-linear-optical microscopy for the integrated and complementary interrogation of plaque tissue constituents and their mutual interactions.

The hybrid multiphoton and optoacoustic microscopy system (MPOM) utilized in this study combines second and third harmonic generation (SHG & THG), two-photon excitation fluorescence (TPEF), brightfield (BF), and optical-resolution optoacoustic microscopy (OAM). All modalities are characterized by a lateral resolution of ~1 μm and a maximum imaging depth of up to ~300 μm. MPOM consists of two separate laser systems, which are based on a common and co-aligned beam path guided consecutively into an inverted microscope. Final image generation is achieved by high-speed raster-scanning of a focused optical excitation beam across the specimen.

We used widefield BF and coarse OAM appearances to schematically subdivide atherosclerotic tissue samples according to discrete compartments. MPOM microscopic imaging demonstrated the label-free visualization of different degrees of interaction among the plaque moieties such as connective tissue, overall cell morphology, and intraplaque blood embeddings within the atheroma. The revealed interactions ranged from intact connective tissue with preserved morphological features and no mutual interaction over coarsely alternating structures of red blood cells (RBCs) and connective tissue combined with a slightly disturbed cell morphology up to a fine interleaving of embedded RBCs and disrupted collagen fibrils along with an advanced degradation of cell morphology.

10064-186, Session PTue

All optical reflection mode dual modality optical resolution photoacoustic and optical coherence microscopy system using an akinetic acoustic sensor

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Photoacoustic microscopy (PAM) and optical coherence tomography (OCT) have both been explored extensively for biomedical applications. With complementary contrast mechanisms, efforts have been made to combine these two optical imaging modalities so that absorption and scattering contrasts are acquired simultaneously. Most dual modality PAM/OCT configurations use piezoelectric transducers. The generally opaque material of the transducers causes difficulties in implementation of the dual modality systems. In our work, a rigid acoustic sensor without deformable membrane or mechanical movement is used. This akinetic sensor features a relatively large central opening, which also serves as the aperture for acoustic wave detection. The acoustically induced optical refractive index change in the central opening can be detected by an interrogation laser beam, whose reflected intensity is modulated by photoacoustic pulses passing through the aperture. Broad bandwidth starting at 500 kHz to tens of megahertz is measured, showing its broadband advantage. High sensitivity is also confirmed by measuring the noise equivalent pressure of the akinetic sensor. A spectral domain OCT (SD-OCT) system is then incorporated into this akinetic PAM system. The transparent central opening of the acoustic sensor permits reflection mode configuration for both modalities. The sample beam of the SD-OCT system and the excitation beam for the PAM system share the same optical path and pass through a microscope objective positioned right above the akinetic sensor, achieving optical resolution. Raster scan of the SD-OCT's sample beam and the PAM's excitation light enables 3D imaging, which is demonstrated by imaging phantoms and animal models in the presented work.

10064-187, Session PTue

Light activated microbubbles for imaging and microsurgery

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In the recent years, imaging and microsurgery techniques based on the photoacoustic effect have been proposed as innovative approaches to manage several pathological conditions such as cancer.

In these applications, the absorption of nanosecond light pulses may trigger a cascade of photothermal and thermoelastic processes that result into the emission of ultrasounds and even the generation of vapor microbubbles. The emission of ultrasounds may be exploited to reconstruct images (photoacustic imaging), while the generation of vapor microbubbles may be used to destroy malignant cells, with the advantage, when compared with traditional optical hyperthermia, of a more limited heat diffusion.

In this context, we developed an experimental set-up to image and destroy malignant cells by photoacoustics that allows us to investigate the generation and manipulation of vapor microbubbles inside malignant cells.

Vapor microbubbles are generated by the interaction between gold nanorods, targetable plasmonic particles with high optical absorption and stability, and the combination of an optical and acoustical activation. In essence optical cavitation is excited with ns duration light pulses in the rarefaction phase of an ultrasound pulse. The synchronize of an optical and acoustical activation allowed to ease the optical requirements to generate microbubbles and to enable their manipulation.

10064-188, Session PTue

Investigation of breast cancer cell lines at different risk level using photoacoustic spectroscopic technique

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Previous study of optical screening of cancer has shown that spectra analysis can be used to distinguish cancer tissue from normal tissue. Normal cells, in general, are characterized by uniformity in size and shape of nuclei (round or oval) and have an ordered morphological structure. But, cancerous nuclei have irregular size and shape, clumps of condensed chromatin, and multiple nucleoli and the prominent nucleoli, marginated nucleoli (location of nucleoli is close to or at the inner nuclear membrane) and multiple nucleoli are criteria for cancer cells.

The aim of the present research is to determine if the photoacoustic induced RF spectroscopy approach is effective to detect changes of cell structure related to different types of cancer cell lines with different risk levels. Different types of cancer cell lines with different risk levels, such as primary tumor carcinoma (MCF-7), moderate metastatic (DU-145), and advanced metastatic (LNCap and PC-3) cell lines, were pump by the tunable near infrared nanosecond laser to explore changes of the RF spectra caused by photacoustic effect. The contributions of different size of cellular partics to the RF spectra from the cell samples were investigated using the Gaussian fitting. The broad bandwidth RF spectra was observed in the advanced metastatic cancer cell lines in comparison with the moderate metastatic and non aggressive cell lines. This work shows the changes of RF spectra obtained from photoacoustic effect spectroscopy may present potential criteria - for detecting different cancer cell lines with different risk levels.

10064-189, Session PTue

Cost effective photoacoustic blood glucose sensor with high sensitivity based on a pulse diode laser

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Continuous and non-invasive glucose monitoring technology can benefit patients with diabetes mellitus by overcoming such current limitations as painful sticking. Photoacoustic (PA) glucose sensing non-invasively monitors the blood glucose level directly from blood vessels. However, several current photoacoustic glucose sensors are limited by their low signal to noise ratio (SNR). Here, we present a novel PA glucose sensor which can monitor glucose with both enhanced detection sensitivity and accuracy. The sensor uses a compact and economical 905 nm-pulsed diode laser with a repetition rate of 100 Hz, with an optical lens group for focusing and collimating. The PA signal is detected by an ultrasonic transducer with a center frequency of 1MHz. In vitro testing involving aqueous glucose solutions validates the photoacoustic technique's sensitivity within a physiological range between 0 and 600 mg/dl, in steps of 30 mg/dl. We observed an increase in peak-to-peak amplitude caused by enhanced absorption of more glucose molecules with glucose concentration. Further, the speed of ultrasound led to a phase shift of the resultant PA signals. The resulting root mean square

error of cross validation (RMSECV) was 14.9 mg/dl. Finally, we successfully monitored the blood glucose concentration of a mouse after an intravenous glucose injection. We believe that this photoacoustic sensor is a promising technology for continuous tracking of physiological blood glucose level in clinical applications.

10064-190, Session PTue

Evaluation of blood glucose concentration measurement using photoacoustic spectroscopy in near-infrared region

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Diabetes, a typical lifestyle-related disease, is an important disease presenting risks of various complications such as retinopathy, kidney failure, and nervous neuropathy. For treating diabetes, regular and continuous self-measurement of blood glucose levels is necessary to maintain blood glucose levels and to prevent complications. Usually, daily measurements are done using invasive methods such as finger-prick blood sampling. Some non-invasive optical techniques have been proposed to reduce pain and a risk of infection, but only a few practical technique exists today.

To realize highly accurate and practical measurement of blood glucose concentrations, the feasibility of a photoacoustic method using near-infrared light was evaluated. A photoacoustic signal from solution of glucose in water (+0–10 g/dl) or equine blood (+0–400 mg/dl) was measured using a hydrophone (9 mm dia.) at 800–1800 nm wavelengths. The relation between the glucose solution concentration and the photoacoustic signal intensity or peak position of the received photoacoustic signal (i.e. sound speed of solutions) was investigated. Results show that the signal intensity and sound speed of the glucose solution increase with the incleased glucose concentration at wavelengths where light absorbance of glucose is high.

For quantitative estimation of the glucose solution concentration, the photoacoustic signal intensity ratio between two wavelengths, at which dependence of the signal intensity on glucose concentration is high and low, was calculated. Results confirmed that the signal intensity ratios increase linearly with the glucose concentration.

These analyses verified the feasibility of glucose level estimation using photoacoustic measurement in the near-infrared region.

10064-191, Session PTue

Biological tissue component evaluation by measuring photoacoustic spectrum

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Photoacoustic imaging has garnered constant attention as a non-invasive modality for visualizing details of the neovascularization structure of tumors, or the distribution of oxygen saturation, which is related to tumor grade. However, photoacoustic imaging is applicable not only for vascular imaging but also for diagnosing properties of various tissues, such as skin or muscle diseases, fat related to arteriosclerosis or fatty liver, cartilage related to arthritis, and fibrous tissues related to hepatitis. The photoacoustic signal intensity is wavelength-dependent and proportional to the absorption coefficient and thermal acoustic conversion efficiency (i.e. Grüneisen parameter) of the target biological tissue.

To ascertain the appropriate wavelength range for biological tissue imaging and evaluating tissue properties, photoacoustic spectra of various tissues (e.g., skin, musculus, and adipose tissue) were measured using a hydrophone (9 mm dia.) at 800–1600 nm wavelengths. Results confirmed that each tissue has a unique photoacoustic spectrum. However, almost all samples have peaks around 1200 nm and 1400–1500 nm at wavelengths where the light absorbance of lipid or water is high. The main components of biological tissues are water, protein, and lipid. Results confirmed that photoacoustic spectra reflect the tissue components well.

To evaluate the feasibility of the tissue characterization using photoacoustic methods, the photoacoustic signal intensity ratio between two wavelength regions as described above were calculated. Signal intensity ratios agreed well with the composition ratio between water and lipid in samples. These analyses verified the feasibility of evaluating tissue properties using photoacoustic methods.

10064-192, Session PTue

Optical-frequency-comb-based ultrasound sensor

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Photo-acoustic imaging is a promising modality for deep tissue imaging with high spatial resolution in the field of biology and medicine. High penetration depth and spatial resolution of the photo-acoustic imaging is achieved by means of the advantages of optical and ultrasound imaging, i.e. tightly focused beam confines ultrasound-generated region within micrometer scale and the ultrasound can propagate through tissues without significant energy loss. To enhance the detection sensitivity and penetration depth of the photo-acoustic imaging, highly sensitive ultrasound detector is greatly desired. In this study, we proposed a novel ultrasound detector employing optical frequency comb (OFC) cavity. Ultrasound generated by the excitation of tightly focused laser beam onto a sample was sensed with a part of an OFC cavity, being encoded into OFC. The spectrally encoded OFC was converted to radio-frequency by the frequency link nature of OFC. The ultrasound-encoded radio-frequency can therefore be directly measured with a high-speed photodetector. We constructed an OFC cavity for ultrasound sensing with a ring-cavity erbium-doped fiber laser. We provided a proof-of-principle demonstration of the detection of ultrasound that was generated by a transducer operating at 10 MHz. Our proposed approach will serve as a unique and powerful tool for detecting ultrasounds for photo-acoustic imaging in the future.

10064-193, Session PTue

Effect of spatial filtering of ultrasound transducers on photoacoustic measurements

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It is intuitively conjectured that the geometrical shape of an ultrasound (US) transducer (TD) as well as US TD's transfer function affects the characteristics of measured photoacoustic (PA) signals. Previously, we theoretically demonstrated with the consideration of the virtual detector concept to a Green function approach that a PA spectrum measured by a spherically focused US TD exhibits resonance frequency peaks. With the experimental verification for the PA resonance peaks, the origin of the PA resonance was discussed as the spatial filtering of the limited measurement field of view of a focused US TD to generated PA waves. In this Proceeding, it is analytically confirmed that a time-domain PA signal derived from the resonant PA spectrum shows a temporal bipolar nature. Also, as one of impacts of the PA resonance, we investigate PA amplitude variation to an absorption coefficient. The results with simulated US TD transfer functions show that a PA signal amplitude decreases as an absorption coefficient of a PA object increases after reaching its maximum value. This phenomenon is totally distinct from the commonly accepted sense that a PA signal amplitude is saturated to an absorption coefficient increase. We analyze the origin of this phenomenon by investigating the relationship between an US TD transfer function and PA resonance frequency.

10064-194, Session PTue

Micromachined silicon acoustic delay line with improved structural stability and acoustic directivity for real-time photoacoustic tomography

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Recent studies have shown that micromachined silicon acoustic delay lines can provide a promising solution to achieve real-time photoacoustic tomography without the need for complex transducer arrays and data acquisition electronics. However, as its length increases to provide longer delay time, the delay line becomes more vulnerable to structural instability due to reduced mechanical stiffness. In addition, the small cross-section area of the delay line results in a large acoustic acceptance angle and therefore poor directivity. To address these two issues, this paper reports the design, fabrication, and testing of a new silicon acoustic delay line enhanced with 3D printed polymer micro linker structures. First, mechanical deformation of the silicon acoustic delay line (with and without linker structures) under gravity was simulated by using finite element method. Second, the acoustic crosstalk and acoustic attenuation caused by the polymer micro linker structures were evaluated with both numerical simulation and ultrasound transmission testing. The result shows that the use of the polymer micro linker structures significantly improves the structural stability of the silicon acoustic delay lines without creating additional acoustic attenuation and crosstalk. In addition, a new tapered design for the input terminal of the delay line was also investigate to improve its acoustic directivity by reducing the acoustic acceptance angle. These two improvements are expected to provide an effective solution to eliminate current limitations on the achievable acoustic delay time and out-of-plane imaging resolution of micromachined silicon acoustic delay line arrays.

10064-195, Session PTue

Quantitative photoacoustic assessment of red blood cell aggregation under pulsatile blood flow: experimental and theoretical approaches

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In this paper we attempt to quantitatively assess red blood cell (RBC) aggregation by high-frequency photoacoustics under pulsatile blood flow. Photoacoustic (PA) radio-frequency parameters such as the spectral slope (SS) and mid-band fit (MBF) are system-independent variables with potential for detecting structural changes in ultrasonic-resolution-PA-imaging.

The pulsatile flow of porcine whole blood at 60 bpm was imaged using the VevoLAZR system (40-MHz-linear-array probe, 700-900 nm illuminations). Power spectra were normalized by reference spectra from non-aggregating, flowing RBC suspensions. The SS and MBF were computed as a function of time and wavelength. The theoretical model approximated spherical aggregates with 2, 3, 4, 5 and 10 RBCs (80% oxygenated, 700 nm illumination). The average power spectra were computed from 100 random distributions of single/aggregated RBCs (40% hematocrit in 50 ? 50 ? 200 μm3 area).

The SS and MBF varied cyclically and were out of phase with one another. The average magnitude of the variation was 0.4 dB/MHz and 4 dB for the SS and MBF, respectively; consistent with simulations. The pulsatile-induced cyclic variation in shear rate effect on RBC aggregation caused the SS to increase as the flow velocity decreased and the MBF to decrease. Simulations showed that both parameters are sensitive to aggregate size. The SS decreased by 0.4 dB/MHz from 700-900 nm; the MBF increased by 10 dB. The trend in MBF resembled oxy-hemoglobin's molar extinction at 80% oxygenation. The SS wavelength-dependence suggests that it could be sensitive to the combined size of oxy/deoxy blood cells which changes as a function of wavelength and aggregation. These quantitative results show potential in tracking flow-induced, RBC structural changes using the frequency of PA signals.

10064-196, Session PTue

Adipocyte property evaluation with photoacoustic spectrum analysis: a feasibility study on human tissues

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Photoacoustic spectrum analysis (PASA) offers potential advantages in identifying optically absorbing microstructures in biological tissues. PASA inherited most of the procedures from ultrasound spectrum analysis (USSA), and may have higher sensitivity to detect the physical variety in soft tissues. As a pathology predictor, adipocyte size is usually adopted to evaluate the condition of obese patient, and can be helpful for assessing the patient response to bariatric surgery. In order to acquire adipocyte size, usually adipose tissue biopsy is performed and histopathology analysis is conducted. The whole procedure is not well tolerated by patients, and is also labor and cost intensive. An unmet need is to quantify and predict adipocyte size in a non-invasive and highly efficient way. This work aims at studying the feasibility to analyze the adipocyte size of human fat tissue using the method of PASA. Both computer simulation and experiments on ex vivo human adipose tissue specimens have been completed. Good correlation between the quantified photoacoustic spectral parameter slope and the average adipocyte size obtained by the gold-standard histology has been established. This initial success suggests that the novel PASA could be developed into a useful tool for clinical management of obesity.

10064-197, Session PTue

Comparison study on the feasibility of photoacoustic power spectrum analysis in osteoporosis detection

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Osteoporosis is a progressive bone disease which is characterized by a decrease in the bone mass and deterioration in bone micro-architecture. In theory, photoacoustic (PA) imaging analysis has potential to obtain the characteristics of the bone effectively. The past study demonstrated that photoacoustic spectral analysis (PASA) method with the qualified parameter slope can provide an objective assessment of bone microstructure and deterioration. In this study, we tried to find the advantages of PASA method over the traditional qualified ultrasound (QUS) method in osteoporosis assessment. Numerical simulations of both PA and ultrasound (US) signal were performed on 3D CT images of trabecular bone with different bone mineral densities (BMDs). Experiments were conducted on pig calcaneal bone model with different BMDs, too. We compared the qualified parameter slope and the broadband ultrasonic attenuation (BUA) coefficient from the PASA and QUS among different bone models, respectively. Both the simulation and experiment results showed that bone with low BMD has a higher slope value and lower BUA value. Furthermore, our results demonstrated that the PASA method could provide increased accuracy results and better contrasts in bone assessment, which means that the PASA is more relevant and sensitive to the bone microstructure than the traditional QUS. As the PA is non-ionizing, non-invasive, and it can get better detection parameter slope than the BUA coefficient PASA method holds potential for clinical diagnosis in osteoporosis and other bone diseases.

10064-198, Session PTue

Ultrasound and photoacoustic dual-modality imaging method for measuring cerebrospinal fluid flow rates using perfluorocarbon nanoparticles

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Cerebral shunts are commonly used to treat hydrocephalus, but have a high failure rate. In this study, we report the feasibility of a safe, cost-effective, and portable imaging approach for measuring CSF flow in shunts: contrast-enhanced dual-modality ultrasound/photoacoustic imaging using laser-activated perfluorocarbon nanodroplets (PFCnDs). Both photoacoustic images and nonlinear harmonic ultrasound images based on plane wave compounding were acquired and a two-dimensional speckle tracking method was employed to track PFCnD behavior and quantify CSF flow in a model shunt system. Our studies demonstrated that PFCnD-augmented US/PA imaging can be used to measure physiologically-relevant flow rates and to assess shunt malfunction.

10064-199, Session PTue

Monte-Carlo-based inversion scheme for 3D quantitative photoacoustic tomography

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The goal of quantitative photoacoustic tomography (qPAT) is to recover maps of the chromophore distributions from multiwavelength images of the initial pressure. Model-based inversions which incorporate the physical processes underlying the photoacoustic (PA) signal generation represent a promising approach. Monte-Carlo models of the light transport are computationally expensive, but provide accurate fluence distributions predictions, especially in the ballistic and quasi-ballistic regimes. Here, we focus on the inverse problem of 3D qPAT of blood oxygenation and investigate the application of the Monte-Carlo method in a model-based inversion scheme. A forward model of the light transport based on the MCX simulator and acoustic propagation modeled by the k-Wave toolbox was used to generate a PA image data set acquired in a tissue phantom over a planar detection geometry. The combination of the optical and acoustic models is shown to account for limited view artefacts. In addition, the errors in the fluence due to, for example, partial volume artefacts and absorbers immediately adjacent to the region of interest are investigated. To accomplish large-scale inversions in 3D, the dimensionality is reduced by applying image segmentation techniques to the initial pressure distribution to extract a limited number of regions with homogeneous optical parameters. Furthermore, prior knowledge of parameter constraints based on physiologically realistic values is applied using an iterative parameter search embedded in a Bayesian estimation framework. Finally, we analyse uncertainty and error constraints in the estimation of optical properties and discuss the feasibility of this approach to recover the blood oxygenation from experimental data.

10064-200, Session PTue

Fluence compensated optoacoustic measurements of blood oxygen saturation in vivo at two optimal wavelengths

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The non-invasive measurement of blood oxygen saturation in blood vessels is a promising clinical application of optoacoustic imaging. However, unknown spatial and spectral distribution of the optical fluence challenges the precise multispectral optoacoustic measurements of blood oxygen saturation. The accuracy of the blood oxygen saturation estimates can be improved by the choice of laser wavelengths. We proposed the numerical method to obtain values of the optimal wavelengths needed for two-wavelengths OA measurements of blood oxygen saturation at various depths. The developed method takes into account the acoustic pressure noise, the error in determined values of the optical scattering and absorption coefficients used for the calculation of the optical fluence, and the thickness of the blood vessel chosen for the blood oxygen saturation measurements. We investigated the error, associated with the use of approximate methods of optical fluence evaluation both within the model tissue and the blood vessel. It is shown that, in conditions of an unknown (or partially known) spatial distribution of fluence at depths of 2 to 8 mm, minimal error in the determination of blood oxygen saturation is achieved at wavelengths of 658±40 nm and 1069±40 nm. At BIOS-2017 we will demonstrate the results of OA in vitro and in vivo measurements of blood oxygen saturation using optimal wavelengths obtained by the developed method.

10064-201, Session PTue

A novel method for photoacoustic signal enhancement: towards utilization of low-cost lasers

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In practice, photoacoustic (PA) waves generated in experiments with low-cost lasers, are weak and measured PA signals are almost buried in noise. Reconstruction of an artifact-free PA image from such noisy measurements requires effective signal processing techniques for denoising the PA signals. Conventional averaging techniques are widely used to increase the signal-to-noise ratio (SNR) of such PA signals but the process is time-consuming,

i.e., in the case of very low SNR measurements, hundreds/thousands of data acquisition epochs needed to provide the required SNR. In this study we propose a novel adaptive denoising method based on adaptive line enhancers (ALE) algorithm to effectively reduce noise in PA signals. Results show that in comparison with averaging techniques, the proposed method significantly increases the SNR of the measurements with much less number of epochs. Consequently, the PA image is constructed with a faster frame rate.

10064-202, Session PTue

Curved array and high-speed photoacoustic tomographic data acquisition system for small animal imaging

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in the PXI system, the chassis is an 8-slot 3U PXIe-1082; the system controller in slot 1 is an embedded PXIe-8135, it can host LabVIEW Real-Time applications with embedded controllers, there's no need for an external PC; the two PXIe peripheral slots in slot 2 and 3 are empty; the system timing slot in slot 4 is PXIe-6674T, it can route external clocks and triggers between PXI Express chassis in order to synchronize data acquisition through external synchronization signal of laser; the hybrid peripheral slots in slot 5 to 8 are FlexRIO hardware, one part of which is high-performance I/O adapter module of NI 5752, the other is FPGA module of PXIe-7965R; the software is LabVIEW 2014 SP1 and windows 7.

DMA transfers are accomplished by using a FIFO architecture, the FIFO is composed of 2 parts that behave as one FIFO. The first part of this FIFO is on the FPGA device, this FIFO uses DRAM on the FPGA device, the second part of the DMA FIFO is on the embedded host controller, this portion of the FIFO uses memory on the host controller, the DMA engine automatically transfers data from the FPGA device DRAM to the embedded host controller memory.

The NI 5752 is a 32-channel digitizer adapter module that can sample on all channels at 50 MS/s with 12-bit resolution, according to the requirement of Photoacoustic tomography reconstruction, we need sample 2600 points, and repeat 20 times. The repeated sampling of data can facilitate to smooth the noise generated in the sampling process and help photoacoustic reconstruction.

10064-203, Session PTue

Optimising probe holder design for sentinel lymph node imaging using clinical photoacoustic system with Monte Carlo simulation

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Photoacoustic tomography is a hybrid imaging modality that combines optical and ultrasound imaging. It is rapidly gaining attention in the field of medical imaging. The challenge is to translate it into a clinical setup. In this work, we report the development of a handheld clinical photoacoustic imaging system. A clinical ultrasound imaging system is modified to integrate photoacoustic imaging with the ultrasound imaging. Hence, light delivery has been integrated with the ultrasound probe. The angle of light delivery is optimized in this work with respect to the depth of imaging. Optimization was performed based on Monte Carlo simulation for light transport in tissues. Based on the simulation results, the probe holders were fabricated using 3D printing. Similar results were obtained experimentally using phantoms. Phantoms were developed to mimic sentinel lymph node imaging scenario. Also, in-vivo sentinel lymph node imaging was done using the same system with contrast agents like methylene blue and indocyanine green up to a depth of 1.5 cm. Results confirm that different light illumination angles are required for different imaging depth to get the highest SNR photoacoustic images. For shallower imaging high light launch angle with respect to the skin is better and for deeper imaging lower light launch angles with respect to skin are necessary. The results also validate that one can use Monte Carlo simulation as a tool to optimize the probe holder design depending on the imaging needs. This eliminates a trial and error approach generally used for designing a probe holder.

10064-204, Session PTue

Pulsed laser diode photoacoustic tomography (PLD-PAT) system for fast in vivo imaging of small animal brain

Paul Kumar Upputuri, Sandeep Kumar Kalva, Mohesh Moothanchery, Manojit Pramanik, Nanyang Technological Univ. (Singapore)

In recent years, high-repetition rate pulsed laser diode (PLD) was used as an alternative to the Nd : YAG lasers for photoacoustic tomography (PAT). The use of PLD makes the overall PAT system, a low-cost, portable, and high frame rate imaging tool for preclinical applications. In this work, we will present a portable in vivo pulsed laser diode based photoacoustic tomography (PLD–PAT) system. The PLD is integrated inside a circular scanning geometry. The PLD can provide near-infrared (~803 nm) pulses with pulse duration ~136 ns, and pulse energy ~1.4 mJ / pulse at 7 kHz repetition rate. The system will be demonstrated for in vivo fast imaging of small animal brain. To enhance the contrast of brain imaging, experiments will be carried out using contrast agents which have strong absorption around laser excitation wavelength. This low-cost, portable small animal brain imaging system could be very useful for brain tumor imaging and therapy.

10064-205, Session PTue

Acousto-optic imaging with an application specific integrated circuit

Jean-Michel Tualle, Kinia Barjean, Eric Tinet, Dominique Ettori, Univ. Paris 13 (France); François Ramaz, Institut Langevin, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (France)

Acousto-Optic Imaging (AOI) is an emerging technique in the field of biomedical optics which combines the contrast allowed by diffuse optical tomography with the resolution of ultrasound (US) imaging. The US wave modulates both the refractive index and the scattering particles position. Under a monochromatic light source this induces a modulation at the US frequency of the speckle pattern that results from multiply scattered light. A main challenge with this technology is to record this very weak modulation, bearing in mind that the speckle grains don't oscillate in phase so that their modulations do not add coherently.

We have already presented the ability of a smart-pixels array sensor to manage such a signal [Optics Letters 40, 705-708, 2015]. A lock-in detection is integrated in each pixel. The pixel's output signal is squared in order to measure an energy and to add pixels outputs in a relevant manner. We have shown how such a system can resolve the scattering medium's properties along the US beam propagation axis using Fourier Transform-AOI [J.O.S.A. A 33, 854-862, 2016]. The purpose of the present work is to show how such a system can record 2D images in an efficient way, and with a low acquisition time, compatible with biomedical applications.

10064-206, Session PTue

Quantitative ultrasound modulated optical tomography with holographic photorefractive detection

Clement Dupuy, Institut Langevin, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (France) and Univ. College London (United Kingdom); Samuel Powell, Terence S. Leung, Univ. College London (United Kingdom); François Ramaz, Institut Langevin, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (France)

The highly scattering nature of biological tissues restricts the achievable spatial resolution of images produced using conventional diffuse optical imaging modalities. One way to improve spatial resolution is to use a multi-wave imaging technique such as acousto-optic imaging, which achieves the contrast of optical methods with the spatial resolution of an arbitrarily focused ultrasound field.

The development of a wavefront adaptive holography detection system using photo-refractive crystals sensitive around the near-infrared window allows measurements to be made with a SNR sufficient to image deep inside highly scattering media. Although this technique is sensitive to both absorption and scattering contrast inside the illuminated area, it is only able to produce qualitative images.

In this work we apply a model-based tomographic reconstruction technique to experimentally acquired acousto-optic images using photorefractive crystal detection in order to recover the optical parameters of interest like the absorption coefficient. Using a simple forward model based on the diffusion approximation of the radiative transport equation and an iterative non-linear reconstruction algorithm, we were able to correctly reconstruct the size and absorption coefficients of several millimeter size absorbing targets embedded in a highly scattering phantom with physiologically appropriate optical parameters. This work is the first step in providing clinically relevant quantitive information regarding the optical properties of biological media using the acoustic-optic technique.

10064-207, Session PTue

A charge amplification approach for photoacoustic tomography (PAT) with parallel acoustic delay line (PADL) arrays

Cheng Fang, Arif Kivanc Ustun, Young Cho, Jun Zou, Texas A&M Univ. (United States)

In the past, we have demonstrated a new photoacoustic tomography (PAT) technique based on parallel acoustic delay line (PADL) arrays. By using a PADL array, multiple-channel PA signals can be received simultaneously with one single-element transducer and single-channel data acquisition (DAQ) electronics. To achieve a wider field of view and higher lateral resolution, a larger number of PADLs will be required. As a result, the single-element transducer must have a larger surface area (therefore a higher static capacitance) to interface with all the PADLs. However, the higher static capacitance will reduce the PA voltage output from the transducer, resulting in lower transducer-amplifier gain and signal-to-noise ratio (SNR). To address this issue, we report a new charge amplification approach for the PADL-enhanced PAT. In contrast to the conventional voltage amplification, the charge amplification offers a constant transducer-amplifier gain for the PA signals regardless of the size of the single-element transducer. First, the charge amplifier was designed, simulated and constructed. Next, the transducer-amplifier gain was characterized by interfacing the charge amplifier with single-element transducers with different element size, respectively. Last, PAT imaging experiments were conducted using a silicon PADL array and the charge amplifier. The imaging results show improved SNR and image quality over the one obtained with conventional voltage amplifier. Therefore, this charge amplification approach could overcome the practical limit on the number of PADLs that can be interfaced with a single-element transducer. It could open new possibilities for PADL-enhanced PAT with large field of view and high lateral resolution.

10064-208, Session PTue

Transparent microring resonator ultrasound detector fabricated by soft nano-imprinting lithography

Hao Li, Xiangfan Chen, Cheng Sun, Hao F. Zhang, Biqin Dong, Northwestern Univ. (United States)

The rapid advance of photoacoustic microscopy (PAM) technology and its applications in clinical diagnosis and biomedical research motivate high lateral/axial imaging resolution and its integration with existing optical imaging modalities. It requires broadband and sensitive ultrasound detectors but obstructing the optical path of the incident laser beam. We have previously developed a novel transparent optical microring resonator (MRR) ultrasound detector, where ultrasonic wave was detected by light-sound interaction and signal was amplified by the underlying optical resonance. We have demonstrated the ultrasound detection bandwidths of over 100 MHz and pressure detection limits of lower than to 10 Pa. The miniaturized size and optical transparency of MRR detectors also enabled easy integration of PA imaging with other existing optical imaging modalities, including endoscopic optical coherence tomography and confocal fluorescence imaging, collectively providing abundant information of biological samples form multi-contrast images.

Despite these unique advantages, MRR ultrasound detectors were fabricated using rather time-consuming and expensive electron-beam lithography (EBL) process. The fabricated MRR ultrasound detectors also need to be individually tested and selected due to the poor reliability of the EBL process, which further increase the fabrication cost. To overcome this issue, we presented a simple and cost efficient soft nanoimprinting lithography process to fabricate MRR ultrasound detectors on transparent substrates. We also characterized the sensitivity and bandwidth of the fabricated detector and performed PA imaging on phantom samples. This work could potentially fill the gap between the nano-fabrication and end-user applications of MRR ultrasound detectors for both clinical applications and biological researches.

10064-209, Session PTue

Utility of ultrasound/photoacoustic imaging for accurate catheter visualization and tracking during endovenous laser ablation

Ayushi Jharia, Keerthana Palani, Yan Yan, Wayne State University (United States); Loay S. Kabbani, Henry Ford Hospital (United States); Mohammad Mehrmohammadi, Wayne State University (United States)

Currently, a common minimally invasive treatment option for patients with varicose veins includes laser ablation. During endovenous laser therapy, ultrasound (US) imaging is often used as gold-standard to help surgeons visualizing and accurate placement of the ablation catheter within the target vessels. However, US imaging has certain limitations such as angular dependency and comet tail artifacts makes it difficult in US imaging to accurately locate the catheter in small perforating veins. In this study, we propose utilizing combined US and Photoacoustic (PA) imaging as a suitable tool to improve the localization of ablation catheter within the tissue. Preliminary results were performed using a large-core, multi-mode fiber optics (diameter of 1000 ?m). A custom-built fiber holder was designed to enable tilting fiber at controlled and desired angles and with respect the incident US beams. The fiber was then placed inside a phantom

consisted of a PVA background and sheep blood and both US and PA images were acquired in different scenarios where fiber was placed at 90 to 30 degrees angel with respect to US incident beam (60 degrees tilting). US and PA image acquisition was performed with a programmable US scanner equipped with a linear array US transducer. Our results indicate while US imaging have difficulties to identify the location of the fiber tip when the fiber is tilted, PA images are very consistent (mean PA signal intensity variation <10%) indicating the independency of PA in locating the fiber tip at different angles.

10064-210, Session PTue

Endocavity ultrasound and photoacoustic imaging: towards enhanced fetal and neonatal monitoring

Yan Yan, Suhail S. Alshahrani, Edgar Hernandez-Andrade, Juri Gelovani, Sonia S. Hassan, Mohammad Mehrmohammadi, Wayne State Univ. (United States)

Intrauterine hypoxia is an unexpected condition during delivery in which the fetus is deprived of an adequate supply of oxygen. Intrauterine hypoxia can put the infants at risk of serious medical conditions including hypoxic ischemic encephalopathy (HIE). Currently, the fetal status during labor is monitored by continuous intrapartum fetal heart rate monitoring (IFHRM), which most of the fetuses with mild or moderate exposure to intrapartum hypoxia will not be identified. However, there is an unmet need for a diagnostic tool which can directly measure the hemoglobin oxygen saturation in fetus brain. Ultrasound (US) and Photoacoustic (PA) imaging have shown to be capable of imaging blood flow and hemoglobin oxygen saturation in blood vessels. While USPA imaging of brain is associated with certain limitations due to the presence of skull, thin and relatively softer skull bone in fetus and neonates and especially the presence of fontanelles allows for accessing the brain for imaging. We have designed and developed an endocavity US and PA imaging probe, consisted of a an endovaginal US transducer (ATL C9-5) and an optimized integrated light delivery system consisted of 18 large core (1000 μm) multimode fibers. Our experimental results indicate the possibility of detecting mimicked blood vessels at large depths (>35 mm) within the porcine tissue. We also demonstrated the ability of the developed probe to measure blood flow (fractional moving blood volume) and hemoglobin oxygen saturation (SO2) through a set of ex vivo experiments using heparinized sheep blood mixed with different concentration of sodium dithionite to mimic different oxygenation level.

10064-211, Session PTue

Array-based ultrasound and photoacoustic tomography for breast cancer imaging

Suhail S. Alshahrani, Yan Yan, Sirisha Kondle, Barrington O'Brian Brown, Wayne State Univ. (United States); Neb Duric, Karmanos Cancer Institute (United States); Mohammad Mehrmohammadi, Wayne State Univ. (United States) and Karmanos Cancer Institute (United States)

Breast cancer is a major health problem in the United States and the world. An estimated 246,660 new, invasive breast cancer cases in females are expected to be diagnosed in the United States in 2016. Mammography, magnetic resonance imaging (MRI), and ultrasound (US) are the major imaging modalities often used to detect breast cancer. Ultrasound imaging shows performance in detecting suspicious breast cancer lesions, but the poor specificity of US imaging limits its diagnostic power in differentiating between benign and malignant breast lesions. In recent years, photoacoustic (PA) imaging has shown great promise in the detection and staging of cancer. Combining PA and US imaging is a strong tool that can provide various sets of information including structure and morphology of the pathologic tissue. In this study, a single array US/PA tomography system has been developed to scan different types of tissue-mimicking phantoms and live animals. In addition, we investigated on optimized light delivery strategies for omni-directional illumination on breast tissue. Our imaging system consisted of a fully digital, programmable US scanner, a tunable pulsed laser source, and a computer-controlled motorized rotation/translation scanning unit that could rotate and translate the objects within the water tank in order to obtain volumetric tomographic images. An FPGA-based control unit was utilized to synchronize the laser and the US acquisition machine, allowing for interleaved acquisition of US and PA frames. The system was tested with calibration phantoms to characterize the resolution and showed to be able to achieve the resolution of 200 μm. Moreover, the tomographic images were acquired in tissue gelatin-based mimicking phantoms with embedded gelatin/ blood inclusions. Our results indicate the ability of the system to acquire co-registered USPA images containing both acoustical and optical contrast and thus providing both anatomical and functional information on the tissue.

10064-212, Session PTue

Iterative image reconstruction in elastic heterogeneous media with application to transcranial photoacoustic tomography

Joemini Poudel, Kenji Mitsuhashi, Thomas P. Matthews, Alejandro Garcia-Uribe, Lihong V. Wang, Mark A. Anastasio, Washington Univ. in St. Louis (United States)

Photoacoustic computed tomography (PACT) is an emerging computed imaging modality that exploits optical contrast and ultrasonic detection principles to form images of the absorbed optical energy density within tissue. The PACT reconstruction problem corresponds to recovering the total absorbed optical density within a tissue sample, from the acoustic waves recorded on a measurement aperture located outside the support of the tissue sample. A major challenge in transcranial PACT brain imaging is to compensate for aberrations in the measured photoacoustic data due to their propagation through the skull. The transmission of ultrasonic waves through the skull induces strong changes to the photoacoustic wavefield through the processes of absorption, scattering, and longitudinal-to-shear wave mode conversion. To properly account for these effects, a wave equation-based inversion method should be employed that can model the heterogeneous elastic properties of the medium. In this work, an iterative image reconstruction method for three-dimensional (3D) transcranial PACT is developed that is based on the elastic wave equation. To accomplish this, a forward model based on a finite-difference time domain discretization of the elastic wave equation is established. Subsequently, gradient-based methods are employed for computing penalized least squares estimates of the total absorbed optical density distribution that produced the measured photoacoustic data. Reconstructed images from both numerical phantoms as well as experimental data are employed to demonstrate the feasibility and effectiveness of the approach.

10064-213, Session PTue

Challenges in optoacoustic temperature imaging

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There are few methods for 3D imaging of temperature for monitoring thermal therapies. Optoacoustic tomography (OAT) could serve as a lower cost, higher frame-rate solution compared with MRI thermometry, which represents the current state of the art. However, a number of challenges must be overcome before OAT thermometry can be employed to estimate temperature in vivo. First, variations in the acoustic properties of the object being imaged can lead to systematic errors in the estimated temperature

distribution when these variations are not accounted for as part of the image reconstruction process. Second, motion artifacts, particularly in the case of small animal imaging, can limit the usefulness of reference images to compensate for the heterogeneous fluence distribution. Third, lack of knowledge of the initial temperature distribution can hinder quantitative estimation of changes in temperature. Fourth, practical imaging geometries that permit concurrent thermal therapy may prevent use of full-coverage detection systems. Here, we detail a multi-stage image reconstruction process that can compensate for variations in the acoustic properties of the object and minimize artifacts due to motion. The methodology is demonstrated through computer simulation studies for a limited view imaging geometry designed to accommodate an existing HIFU thermal therapy system. We provide methods for estimating temperature changes when the initial temperature distribution is both known and unknown. We also discuss additional challenges and areas for future work.

10064-81, Session 13

Photoacoustic physio-chemical analysis for prostate cancer diagnosis

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Photoacoustic physio-chemical analysis (PAPCA) is a recently developed technology capable of simultaneously quantifying the content of molecular components and the corresponding microarchitectures in biological tissue. We have successfully quantified the diagnostic information in livers with PAPCA. In this study, we implemented PAPCA to the diagnosis of prostate cancers. 4 human prostates were scanned ex vivo. The PA signals from normal and cancerous regions in the prostates were acquired by an interstitial needle PA probe. A total of 14 interstitial measurements, including 6 within the normal regions and 8 in the cancerous regions, were acquired. The observed changes in molecular components, including lipid, collagen and hemoglobin were consistent with the findings by other research groups. The changes were quantified by PA spectral analysis (PASA) at wavelengths where strong optical absorption of the relevant molecular components was found. Statistically significant differences among the PASA parameters were observed (p=0.025 at significance of 0.05). A support vector machine model for differentiating the normal and cancerous tissue was established. With the limited number of samples, an 85% diagnostic accuracy was found. The diagnostic information in the PCPCA can be further enriched by targeted optical contrast agents visualizing the microarchitecture in PCa tissues. F3 PAA-PEG nanoparticles was employed to stain the PCa cells in a transgenic mouse model, in which the microarchitectures of normal and cancerous prostate tissues are comparable to that in human. Statistically significant differences were observed between the contrast-enhanced normal and cancerous regions (p=0.038 at a significance of 0.05).

10064-82, Session 13

Rate-of-change contrast for multiplexed background-free photoacoustic and fluorescent imaging of photoswitchable chromoproteins

Ryan K. Chee, Yan Li, Robert J. Paproski, Robert E. Campbell, Roger J. Zemp, Univ. of Alberta (Canada)

Molecular photoacoustic imaging is hindered by hemoglobin background signal. Photoswitchable chromoproteins can be used to obtain images with significantly reduced background signal. Molecular imaging of multiple biological processes via multiple chromoprotiens is difficult due to overlapping imaging spectra. Using a new rate-of-change imaging methodology, we can obtain molecular images with multiple chromoprotiens with overlapping imaging spectra. We also present a new photoswitchable chromoprotein, GAF2, which is significantly smaller than the BphP1 which has shown promise for photoswitchable photoacoustic imaging [Yao et al., Nat. Meth. 13, 67–73 (2016)].

We use BphP1 and GAF2 with photoacoustic (Vevo LAZR, Fujifilm Visualsonics Inc) and fluorescence (In vivo Xtreme, Bruker) imaging systems to show background-free multiplexed images. We image before, after, and during photoconversion to obtain background-free rate-of-change images and compare our results to difference imaging and spectral demixing. After phantom imaging, we inject mice with different chromoprotein-expressing E. coli bacteria to show multiplexed images of bacterial infections.

We show distinguishable differences in the rate-of-change between GAF2 and BphP1. We obtain rate-of-change feasibility images and in vivo images in mice showing the ability to differentiate between GAF2 and BphP1 even though they are spectrally similar. We photoconvert both GAF2 and BphP1 using 550nm and 735nm light. Phantom studies suggest a 10-20dB improvement in the rate-of-change and difference images in comparison to images with background. Multiplexed background-free molecular imaging using chromoproteins could prove to be a promising new imaging methodology especially when combined with spectral demixing.

10064-83, Session 13

Photoacoustic imaging of intestinal strictures: microscopic and macroscopic assessment in vivo

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The pathology of Crohn's disease (CD) is characterized by obstructing intestinal strictures because of inflammation (with high levels of hemoglobin), fibrosis (high levels of collagen), or a combination of both. Inflammatory strictures are medically treated. Fibrotic strictures have to be removed surgically. The accurate characterization of the strictures is therefore critical for the management of CD. Currently the comprehensive assessment of a stricture is difficult, as the standard diagnostic procedure, endoscopic biopsy, is superficial and with limited locations as well as depth. In our previous studies, photoacoustic imaging (PAI) has recovered the layered architectures and the relative content of the molecular components in human and animal tissues ex vivo. This study will investigate the capability of multispectral PAI in resolving the architecture and the molecular components of intestinal strictures in rats in vivo. PA images at 532, 1210 and 1310 nm targeting the strong optical absorption of hemoglobin, lipid and collagen were acquired using two approaches. A compact linear array, CL15-7, was used to transcutaneously acquire PA signals generated by the a fiber optics diffuser positioned within the inner lumen of the strictures. Another approach was to use an endoscopic capsule probe for acoustic resolution PA microscopy. The capsule probe is designed for human and therefore cannot fit into rat colon. The inner surface of the intestinal stricture was exposed and the probe was attached to the diseased location for imaging. The findings in PA images were confirmed by histology results.

10064-85, Session 13

A strategy to measure electrophysiological changes with photoacoustic imaging

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Photoacoustic imaging is an emerging technology capable of both functional and structural biological imaging. Absorption and scattering in tissue limit the penetration depth of conventional microscopy techniques to <1mm. Photoacoustic imaging however, can offer high-resolution and contrast at depths of several centimeters. Though functional imaging of endogenous contrast agents, such as hemoglobin, is widely implemented, currently photoacoustic imaging is unable to functionally report electrophysiological changes within cells. We aim to develop photoacoustic contrast agents to fulfill this need. Cells throughout the brain and body create electrical signals using ion channel proteins. These proteins undergo structural changes to regulate the flux of salt ions into the cell. We have recently developed ion channel activity tracers that dissociate from ion channels after the protein changes structure. By conjugating the tracer to dyes that are sensitive to changes in their chemical environment, we can detect tracer dissociation and therefore ion channel activity. We are exploring whether a similar mechanism can create photoacoustic signal intensity changes. To test if the environmental sensitivity of the dye is photoacoustically distinguishable, we imaged the dye in different solvent backgrounds. We report that manipulation of the chemical environment of the contrast dye results in robust changes in photoacoustic properties. We are working to capture photoacoustic signal changes that occur when ion channel proteins activate using live cell imaging. This technology could permit photoacoustic imaging of electrophysiological dynamics in deep tissue, such as the brain. Further optimization of this technology could lead to concurrent imaging of neural activity and hemodynamic responses, a crucial step towards understanding neurovascular coupling in the brain.

10064-86, Session 13

Copper sulfide nanodisk as photoacoustic contrast agent for ovarian tumor detection

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Screening for ovarian cancer has poor specificity and sensitivity, and photoacoustic imaging can potentially offer increase tumor contrast to improve the positive predictive index. Copper sulfide (CuS) nanodisks is a powerful photoacoustic contrast agent due to their directionally-localized surface plasmon resonance in the near infrared region. We prepared CuS nanodisks via a solvent-based synthesis followed by surface modification of poly(ethylene glycol) methyl ether thiol (PEG-thiol, Mw=5000, diameter= 18.2 ± 1.8 nm, thickness= 3.98 ± 0.85 nm). These CuS nanodisks were stable in phosphate buffered saline (PBS) for two weeks and offered 2.3-fold better and more stable signal than gold nanorod (GNR) at 700 nm (near maximum GNR absorption) at a concentration of 1.25 nM. Nude mice with 10-week-old subcutaneous ovarian tumors (SKOV3) were intravenously injected with 200 μL 150 nM CuS nanodisks. The tumor signal was 6.6-fold higher in these CuS-treated animals than in the control injected with PBS (both cohorts imaged at 920 nm, p<0.01). We then compared the photoacoustic spectra of tumors with the photoacoustic spectrum of CuS nanodisks and confirmed that the enhanced photoacoustic signals came from CuS. Future work will evaluate this novel shape versus other geometries to further optimize the accumulation of CuS nanoparticles in ovarian tumor.

10064-87, Session 13

Highly sensitive magneto-motive photoacoustic imaging using stably doped conducting polymer nanoshells

Soon Joon Yoon, Junwei Li, Bao-Yu Hsieh, Wanyi Tai, Xiaohu Gao, Matthew O'Donnell, Univ. of Washington (United States)

In recent years, photoacoustic (PA) imaging has been widely investigated as a promising molecular imaging modality in conjunction with highly sensitive nanoprobes. However, the specificity of PA imaging under in-vivo conditions must be improved due to background signals from tissue. In this study, we investigated a new type of multimodal magneto-optically coupled core-shell magnetic nanoparticle with a stably doped conductive polymer shell (MNP-bPANI(polyaniline)) in a biological environment. The sizes of the core magnetic nanoparticle and MNP-bPANI particle are 38 nm / 68 nm in diameter, respectively. These nanoparticles feature high colloidal stability and a broad near-infrared (NIR) spectrum absorbing polyaniline polymer shell. We have tested them using our magneto-motive photoacoustic and ultrasound (PAUS) imaging system with cyclic excitations in a mouse tumor model in-vivo. To grow a tumor in mice, LNCaP prostate tumor cells was injected subcutaneously and MNP-bPANI particles were administered via intratumoral injection after the tumor reached a certain size. By synchronizing local displacement calculated by ultrasound speckle tracking with the magnetic excitation, the tumor site moving coherently was distinguished from background static signals and signal moving incoherently. The results suggest that this technique can improve the specificity of PA imaging by eliminating unwanted background signal originating from either physiological motion or endogenous contrast agents such as hemoglobin, melanin, and lipid. In addition, these particles absorb over a broad optical spectrum and are naturally biocompatible, key features for ultimate clinical translation.

10064-88, Session 13

Deep-tissue photoacoustic imaging of cell viability

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For emerging tissue-engineering applications, transplants, and cell-based therapies it is important to assess cell viability and function in vivo in deep tissues. Bioluminescence and fluorescence methods are poorly suited to deep monitoring applications with high resolution and require genetically-engineered reporters which are not always feasible. We report on a method for imaging cell viability using deep, high-resolution photoacoustic imaging. We use an exogenous dye, Resazurin, itself weakly fluorescent until it is reduced from blue to a pink color with bright red fluorescence. Upon cell death fluorescence is lost and an absorption shift is observed. The irreversible reaction of resazurin to resorufin is proportional to aerobic respiration. We detect colorimetric absorption shifts using multispectral photoacoustic imaging and quantify the fraction of viable cells. SKOV-3 cells with and without ±80oC heat treatment were imaged after Resazurin treatment. High 575nm:620nm ratiometric absorption and photoacoustic signals in viable cells were observed with a much lower ratio in low-viability populations.

10064-89, Session 13

Polypyrrole coated phase-change contrast agents for sono-photoacoustic imaging

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Ultrasound, Univ. of Washington (United States); Matthew O'Donnell, Lilo D. Pozzo, Univ. of Washington (United States)

A new light and sound sensitive nanoemulsion contrast agent is presented. The agents feature a low boiling point liquid perfluorocarbon core and a broad light spectrum absorbing polypyrrole (PPy) polymer shell. The PPy coated nanoemulsions can reversibly convert from liquid to gas phase upon cavitation of the liquid perfluorocarbon core. Cavitation can be initiated using a sufficiently high intensity acoustic pulse or from heat generation due to light absorption from a laser pulse. The emulsions can be made between 150 and 350 nm in diameter and PPy has a broad optical absorption covering both the visible spectrum and extending into the near-infrared spectrum (peak absorption ~1053 nm).

The size, structure, and optical absorption properties of the PPy coated nanoemulsions were characterized and compared to PPy nanoparticles (no liquid core) using dynamic light scattering, ultraviolet-visible spectrophotometry, transmission electron microscopy, and small angle X-ray scattering. The cavitation threshold and signal intensity were measured as a function of both acoustic pressure and laser fluence. Overlapping simultaneous transmission of an acoustic and laser pulse can significantly reduce the activation energy of the contrast agents to levels lower than optical or acoustic activation alone. We also demonstrate that simultaneous light and sound cavitation of the agents can be used in a new sono-photoacoustic imaging method, which enables greater sensitivity than traditional photoacoustic imaging.

10064-90, Session 14

Photoacoustic super-resolution microscopy using blind structured speckle illumination

Peter Burgholzer, Research Ctr. for Non Destructive Testing GmbH (Austria); Todd W. Murray, Univ. of Colorado Boulder (United States); Markus Haltmeier, Univ. of Innsbruck (Austria); Thomas Berer, Elisabeth Leiss-Holzinger, Research Ctr. for Non Destructive Testing GmbH (Austria)

We present an imaging method that uses the random optical speckle patterns that naturally emerge as light propagates through strongly scattering media as a structured illumination source for photoacoustic imaging. Our approach, termed blind structured illumination photoacoustic microscopy (BSIPAM), was inspired by recent work in fluorescence microscopy where super-resolution imaging was demonstrated using multiple unknown speckle illumination patterns. We extend this concept to the multiple scattering domain using photoacoustics (PA), with the speckle pattern serving to generate ultrasound. The optical speckle pattern that emerges as light propagates through diffuse media provides structured illumination to an object placed behind a scattering wall. The photoacoustic signal produced by such illumination is detected using a focused ultrasound transducer. We demonstrate through both simulation and experiment, that by acquiring multiple photoacoustic images, each produced by a different random and unknown speckle pattern, an image of an absorbing object can be reconstructed with a spatial resolution far exceeding that of the ultrasound transducer. We experimentally and numerically demonstrate a gain in resolution of more than a factor of two by using multiple speckle illuminations. The variations in the photoacoustic signals generated with random speckle patterns are utilized in BSIPAM using a novel reconstruction algorithm. Exploiting joint sparsity, this algorithm is capable of reconstructing the absorbing structure from measured PA signals with a resolution close to the speckle size.

10064-91, Session 14

Compressed sensing in photoacoustic imaging and application for planar detection geometries

Thomas Berer, Peter Burgholzer, Research Ctr. for Non Destructive Testing GmbH (Austria); Markus Haltmeier, Univ. of Innsbruck (Austria)

Increasing the imaging speed is a central aim in photoacoustic tomography. Increasing the imaging speed is even more crucial for optical detection schemes where an optical interrogation beam is scanned along a planar detection surface and the ultrasonic waves are recorded at each position sequentially. In this work we address this issue using techniques of compressed sensing. We demonstrate that the number of measurements can significantly be reduced by allowing general linear measurements instead of point wise pressure values. A main requirement in compressed sensing is the sparsity of the unknowns to be recovered. Sparsity of the pressure wave as a function of space and time is not valid directly. Therefore, we introduce the concept of sparsifying temporal transforms for three-dimensional photoacoustic imaging, which allows obtaining theoretical recovery guarantees. We present reconstruction results for simulated as well as for experimentally obtained data. The latter are acquired by using a non-contact photoacoustic imaging system. The simulated and the experimental data verify that the proposed compressed sensing scheme allows a significant reduction of the number of spatial measurements without sacrificing the spatial resolution.

10064-92, Session 14

Improving image reconstruction of bioluminescence imaging using a priori information from ultrasound imaging

Baptiste Jayet, Junaid Ahmad, The Univ. of Nottingham (United Kingdom); Shelley L. Taylor, The Univ. of Birmingham (United Kingdom); Philip J. Hill, The Univ. of Nottingham (United Kingdom); Hamid Dehghani, The Univ. of Birmingham (United Kingdom); Stephen P. Morgan, The Univ. of Nottingham (United Kingdom)

Bioluminescence imaging (BLI) is a commonly used imaging modality in biology to study cancer in vivo in small animals. Images are generated using a camera to map the optical fluence emerging from the studied animal, then a numerical reconstruction algorithm is used to locate the sources and estimate their sizes. However, due to the strong light scattering properties of biological tissues, the resolution is very limited (around a few millimetres). Therefore obtaining accurate information about the pathology is complicated. We propose a combined ultrasound/optics approach to improve accuracy of these techniques. In addition to the BLI data, an ultrasound probe driven by a scanner is used for two main objectives. First, to obtain a pure acoustic image, which provides structural information of the sample. And second, to alter the light emission by the bioluminescent sources embedded inside the sample, which is monitored using a high speed optical detector (e.g. photomultiplier tube).

We will show that this last measurement, used in conjunction with the ultrasound data, can provide accurate localisation of the bioluminescent sources. This can be used as a priori information by the numerical reconstruction algorithm, greatly increasing the accuracy of the BLI image reconstruction as compared to the image generated using only BLI data.

10064-93, Session 14

Imaging multi-scale dynamics in vivo with spiral volumetric optoacoustic tomography

Xosé Luís Deán-Ben, Thomas F. Fehm, Steven J. Ford, Sven Gottschalk, Daniel Razansky, Helmholtz Zentrum München GmbH (Germany)

Imaging dynamics in living organisms is essential for the understanding of biological complexity. While multiple imaging modalities are often required to cover both microscopic and macroscopic spatial scales, dynamic phenomena may also extend over different temporal scales, necessitating the use of different imaging technologies based on the trade-off between temporal resolution and effective field of view. Optoacoustic (photoacoustic) imaging has been shown to offer the exclusive capability to link multiple spatial scales ranging from organelles to entire organs of small animals. Yet, efficient visualization of multi-scale dynamics remained difficult with state-of-the-art systems due to inefficient trade-offs between image acquisition and effective field of view. Herein, we introduce a spiral volumetric optoacoustic tomography (SVOT) technique that provides spectrally-enriched high-resolution optical absorption contrast across multiple spatio-temporal scales. We demonstrate that SVOT can be used to monitor various in vivo dynamics, from video-rate volumetric visualization of kinetics and motion in whole organs to high-resolution imaging of longitudinal dynamics at the whole body level. The multi-scale dynamic imaging capability thus emerges as a powerful and unique feature of the optoacoustic technology that adds to the multiple advantages of this technology for structural, functional and molecular imaging.

10064-94, Session 14

Weighted synthetic aperture focusing for optoacoustic microscopy with scanning illumination and detection

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Scanning optoacoustic microscopy operates in two distinct regimes – optical resolution microscopy relies on a focused illumination and acoustic resolution microscopy that forms images by focusing the received acoustic field. Recently, a number of approaches have been proposed that combine those two modes of operation to create a highly scalable technique that can image at multiple penetration scales by gradually exchanging microscopic optical resolution in superficial tissues with ultrasonic resolution at diffuse (macroscopic) depths. However, scanning microscopy schemes commonly employ acquisition geometries that impede the use of synthetic aperture techniques to achieve meaningful images due to non-stationary illumination patterns and strong non-uniformity of the excitation light field.

Here we present a Weighted Synthetic Aperture Focusing Technique (W-SAFT) as a universal framework that effectively accounts for the non-uniform distribution of both the excitation light field and spatial sensitivity field of the detector. As a result, W-SAFT maintains optical resolution performance at superficial depths while improving the acoustic resolving capacity for deeper tissues. The dynamic range of the optoacoustic data is compressed using a general fluence decay term applied to the W-SAFT operator, allowing a more uniform visualization of the entire imaged volume. Our three-dimensional algorithm makes use of the sample's surface to account for the heterogeneity produced when scanning a finite-size light beam. We tested a GPU implementation of W-SAFT with numerical simulations and showcase its performance on experimental data acquired from targets embedded in tissue mimicking phantoms.

10064-95, Session 14

Acoustic resolution photoacoustic Doppler flowmetry using a transducer array: optimising processing for velocity contrast

Thore Mainart Bücking, Univ. College London (United Kingdom); Pim J. van den Berg, Univ. Twente (Netherlands); Stavroula Balabani, Univ. College London (United Kingdom); Wiendelt Steenbergen, Univ. Twente (Netherlands); Paul C. Beard, Joanna Brunker, Univ. College London (United Kingdom)

Acoustic Resolution Photoacoustic Flowmetry (AR-PAF) is a novel imaging modality that has the potential to image microvascular blood flow in deep tissue. This could potentially be used for monitoring disease progression of diabetes, sickle cell anaemia, and other pathologies which affect blood flow on the microvascular scale. Despite successful demonstration using phantoms, it has proved extremely challenging to implement the AR-PAF technique to measure the flow of whole blood. Recent success using a single element transducer with a centre frequency of 30 MHz is encouraging but may appear counter-intuitive since, when using this frequency, the detected ultrasound wavelength is much larger than the red blood cell diameter (around 50 μm compared to 8 μm) and the mean spacing between individual red blood cells. While potential explanations for this apparent contradiction have been put forward, a full understanding of the contrast mechanism is still lacking. In order to gain further understanding of the PAF signal, we employed a transducer array and measured flow of whole blood in a polyethylene tube of 0.58 mm diameter and using a detection centre frequency of 15 MHz. We present successful AR-PAF measurements at physiological flow speeds, which is the first demonstration of a 2D AR-PAF scan using whole blood - a major milestone towards implementing the technique in a clinical setting. A direct comparison of AR-PAF using a single element transducer and a transducer array is made by employing an in silico beamforming technique. These insights will direct future experimental design, paving the way towards the detection of clinically relevant markers in microvascular blood flow.

10064-96, Session 14

Fast sparse recovery and coherence factor weighting in optoacoustic tomography

Hailong He, Jaya Prakash, Andreas Buehler, Vasilis Ntziachristos, Helmholtz Zentrum München GmbH (Germany)

A particular challenge in optoacoustic tomography is the implementation of limited-view projections, i.e. cases where 360-degree projections are not available. This could be the case for example in imaging large volumes (whole-animals or humans), whereby access is afforded only from one side of the tissue, in analogy to ultrasound imaging. Limited-view implementations typically results in lower image fidelity and a larger number of artifacts compared to 360-degree view datasets. Nevertheless, sparsity based algorithms were shown to perform better with limited view dataset, compared to Tikhonov based reconstructions, albeit at a higher computational cost. Moreover, sparse recovery based methods may amplify noise in limited-data scenarios. In this paper, we propose an improvement of the fast converging Split Augmented Lagrangian Shrinkage Algorithm (SALSA) method based on least square QR (LSQR) inversion for improving the reconstruction speed. We further show image fidelity improvement when using a coherence factor to weight the reconstruction result. Phantom and in-vivo measurements demonstrate that the accelerated SALSA method with coherence factor weighting (ASALSA-CF) offers images of reduced artifacts and faster convergence compared to existing sparse recovery methods. The implication of the proposed reconstruction method could be in its utility for limited view datasets compared to conventional model-based methods and much faster reconstruction than original sparse methods.

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10065-1, Session 1

Involvement of stress signaling networks in PDT-mediated cell killing and immune response induction *(Invited Paper)*

Mladen Korbelik, British Columbia Cancer Agency (Canada)

Some types of cancer therapy work by inflicting a localized traumatic stress, such as oxidative or thermal stress induced by photodynamic therapy (PDT) and photothermal laser therapy, respectively. This mode of insult provokes in targeted tumor cells homeostatic evolutionary well-preserved protection mechanisms. These activities are operated by signaling networks formed by integrated stress response (ISR) and associated unfolded protein response (UPR). The most conspicuous stressor is misfolded protein accumulated at elevated levels in endoplasmic reticulum (ER). Its presence is detected by sensor kinases whose activation leads to the engagement of transcription factors translocating into the nucleus to regulate expression of ISR and UPR target genes. A major target of sensor kinases is the eukaryotic initiation factor eIF2 whose recruitment leads to global translation attenuation with immediate effects on short half-life proteins. Concomitantly, there is an upregulation of certain transcription factors (e.g. ATF4) that activate anti-oxidative stress genes. If proteostasis cannot be re-established due to severity of adverse effects, the adaptive phase of stress response that promotes cell survival turns into lethal phase promoting cell death, presumably as a protection of the organism from quack cells displaying misfolded proteins. Since these coordinated cytoprotective programs are also triggered by pathogens, they are intimately linked with innate and immune responses and are instrumental in the induction of immunogenic cells death (ICD). In our studies, a series of selective inhibitors of elements of stress signaling pathways were employed to uncover novel therapeutic targets in cellular stress-inducing cancer treatments.

10065-2, Session 1

Stimulation of anti-tumor immune response after photodynamic therapy for cancer *(Invited Paper)*

Michael R. Hamblin, Wellman Ctr. for Photomedicine (United States)

Photodynamic therapy (PDT) has been used as a cancer therapy for forty years but has not yet advanced to a mainstream cancer treatment. Although PDT has been shown to be an efficient photochemical way to destroy local tumors by a combination of non-toxic dyes and harmless visible light, it is its additional effects in mediating the stimulation of the host immune system that gives PDT a great potential to become more widely used. Although the stimulation of tumor-specific cytotoxic T-cells that can destroy distant tumor deposits after PDT has been reported in some animal models, it remains the exception rather than the rule. This realization has prompted several investigators to test various combination approaches that could potentiate the immune recognition of tumor antigens that have been released after PDT. Some of these combination approaches use immunostimulants including various microbial preparations that activate Toll-like receptors and other receptors for pathogen associated molecular patterns. Other approaches use cytokines and growth factors whether directly administered or genetically encoded. A promising approach targets regulatory T-cells. We believe that by understanding the methods employed by tumors to evade immune response and neutralizing them, more precise ways of potentiating PDT-induced immunity can be devised.

10065-3, Session 1

Structural and functional imaging for vascular targeted photodynamic therapy *(Invited Paper)*

Buhong Li, Fujian Normal Univ. (China); Xuelin Chen, Fujian Normal Univ. (China) and Fujian Univ. of Technology (China); Lisheng Lin, Fujian Normal Univ. (China); Defu Chen, Ying Gu, Chinese PLA General Hospital (China)

Vascular targeted photodynamic therapy (V-PDT) has been widely used for the prevention or treatment of vascular-related diseases, such as localized prostate cancer, wet age-related macular degeneration, port wine stains, esophageal varices and bleeding gastrointestinal mucosal lesions. In this talk, the fundamental mechanisms of vascular responses during and after V-PDT will be introduced. Based on the V-PDT treatment of blood vessels in dorsal skinfold window chamber model, the structural and functional imaging, which including white light microscopy, laser speckle imaging, singlet oxygen luminescence imaging, and fluorescence imaging for evaluating vascular damage will be presented, respectively. The results indicate that vessel constriction and blood flow dynamics could be considered as the crucial biomarkers for quantitative evaluation of vascular damage. In addition, future perspectives of non-invasive optical imaging for evaluating vascular damage of V-PDT will be discussed.

10065-4, Session 1

Photothermal therapy combined with dinitrophenyl hapten for the treatment of late stage malignant melanoma *(Invited Paper)*

Xiaosong Li, Nan Du, Chinese PLA General Hospital (China); Haijun Li, The First Cadre's Sanatorium Health Center of Beijing Military Area Command (China); Shan Long, Dianjun Chen, Chinese PLA General Hospital (China); Feifan Zhou, University of Central Oklahoma (United States); Yan Fu, Fangfang Yao, Chinese PLA General Hospital (China); Wei Chen, University of Central Oklahoma (United States)

To evaluate the efficacy and safety of photothermal therapy combined with dinitrophenyl hapten (DNP) for patients with malignant melanoma (MM). Patients with pathology confirmed stage III or IV MM were enrolled. Seventy-two patients were randomized into two groups, DNP alone group (n=32) and DNP plus photothermal therapy group (n=32). The results showed that patients in the combination treatment group had longer median progression-free survival time (19.0m vs. 12.0m, p=0.007). No severe adverse events were observed in both groups. Thus, this may represent a new therapeutic strategy for patients with unresectable, advanced MM.

10065-5, Session 1

Near-infrared imaging guided nanoparticles delivering photoactivated therapeutics

Tymish Y. Ohulchanskyy, Shenzhen Univ. (China) and Univ. at Buffalo (United States); Junle Qu, Shenzhen Univ.

(China); Ravindra K. Pandey, Roswell Park Cancer Institute (United States)

The ability of light to penetrate a tissue is a key to diagnostic (e.g., optical bioimaging) and therapeutic (e.g., light induced therapy) biophotonic applications. Use of the optical "tissue transparency window" in the near-infrared (NIR) range (~700-1000 nm) allows for noninvasive optical bioimaging of small animals in biomedical research, permitting for the imaging guided development of light induced therapy and drug delivery. In addition to this conventional NIR window (NIR-I), other optical windows have recently been identified for ~1000-1350nm (NIR-II) and ~1550-1800 nm (NIR-III or short-wave infrared, SWIR), allowing researchers to benefit from the reduced tissue scattering and autofluorescence in these spectral regions and achieve optical imaging of deeper tissues with better resolution. This talk will present our work on development of optical probes for imaging in NIR-I, II, and III regions, in conjunction with light activated therapy. We have been developing multifunctional molecular and nanostructured materials for NIR imaging guided photoactivated therapy. The photoactive agents, which will be presented, embrace small organic molecules and polymers, silica-based nanoparticles, rare-ion doped nanophosphors, plasmonic nanoparticles, as well as their hybrids and combinations. The presentation will include examples of applications of the nanoparticles as optically traceable agents allowing for "see and treat" approach with photodynamic and photothermal therapies of cancer. The capability of nanoplatforms to be incorporated with other imaging modalities along with the optical one will be deliberated. The talk will conclude with a discussion on challenges in near-infrared optical bioimaging and light induced therapy using NIR-active molecular and nanoparticular agents.

10065-6, Session 2

Near infrared photoimmunotherapy rapidly elicits specific host immunity against cancer cells *(Invited Paper)*

Hisataka Kobayashi, National Cancer Institute (United States)

Near infrared photoimmunotherapy (NIR-PIT) is a new molecularly-targeted cancer photo-therapy based on conjugating a near infrared silica-phthalocyanine dye, IR700, to a monoclonal antibody (mAb) targeting cell-surface molecules. When exposed to NIR light, the conjugate induces a highly-selective necrotic/immunogenic cell death (ICD) only in target-positive, mAb-IR700-bound cancer cells. This cell death occurs as early as 1 minute after exposure to NIR light. Meanwhile, immediately adjacent target-negative cells are unharmed. Dynamic 3D-microscopy of live tumor cells undergoing NIR-PIT showed rapid swelling in treated cells immediately after light exposure, followed by irreversible morphologic changes such as bleb formation, and rupture of vesicles within several minutes. Furthermore, biological markers of ICD including relocation of HSP70/90 and calreticulin, and release of ATP and High Mobility Group Box 1 (HMGB1), were clearly detected immediately after NIR-PIT. When NIR-PIT was performed in a mixture of cancer cells and immature dendritic cells, maturation of immature dendritic cells was strongly induced rapidly after NIR-PIT. Alternatively, NIR-PIT can also target negative regulatory immune cells such as Treg only in the tumor bed. Treg targeting NIR-PIT against CD25 can deplete >80% of Treg in tumor bed within 20 min that induces activation of tumor cell-specific CD8+-T and NK cells within 1.5 hour, and then these activated cells killed cancer cells in local tumor within 1 day and also in distant tumors of the same cell origin within 2 days. In summary, cancer cell-targeting and immuno-suppressor cell-targeting NIR-PITs effectively induce innate and acquired immunity specifically against cancer cells growing in patients, respectively.

10065-7, Session 2

Laser immunotherapy for metastatic pancreatic cancer *(Invited Paper)*

Feifan Zhou, Univ. of Central Oklahoma (United States)

Pancreatic cancer is an extremely malignant disease with high mortality rate. Currently there is no effective therapeutic strategy for highly metastatic pancreatic cancers. Laser immunotherapy (LIT) is a combination therapeutic approach of targeted phototherapy and immunotherapy, which could destroy treated primary tumors with elimination of untreated metastases. LIT affords a remarkable efficacy in suppressing tumor growth in pancreatic tumors in mice, and results in complete tumor regression in many cases. LIT could synergize targeted phototherapy and immunological effects of immunoadjuvant, which represent a promising treatment modality to induce systemic antitumor response through a local intervention, paving the way for the treatment of highly metastatic pancreatic cancers.

10065-8, Session 2

Immunotherapy for advanced solid tumors

Mark F. Naylor M.D., Baylor Scott & White Health (United States)

Immunologic therapy (immunotherapy) has been demonstrated to be the best way to treat advanced melanoma, the prototypical chemotherapy-resistant solid tumor. What remains to be established is the efficacy and benefit of immunotherapy for other tumors that are at least partly responsive to chemotherapy. Breast cancer, partially responsive to traditional chemotherapy, is one of the most common of the solid malignancies. We believe that breast cancer patients, like melanoma patients, will benefit from the application of immunotherapy techniques. We are currently undertaking a study of InCVAX (a laser-based immunostimulation technique) for the treatment of advanced breast cancer patients in Peru, and hope to show that this immunologic treatment can reduce tumor burden, extend survival, and do so in a cost-effective way that has less impact on quality of life compared with traditional chemotherapy for advanced breast cancer.

10065-9, Session 2

InCVAX, a novel in situ autologous cancer vaccine

Samuel Siu Kit Lam, Immunophotonics, Inc. (United States); Wei R. Chen, Univ. of Central Oklahoma (United States)

Cancer immunotherapy is the concept of harnessing our own immune system to fight against cancer cells. The most attractive features of immunotherapy include relatively low toxicities compared to traditional therapies (surgery, chemotherapy and radiation), the possibility of eliminating distant metastases and the potential of preventing relapses. After decades of research, its therapeutic efficacy has finally been recognized and a number of approaches has been approved by the FDA over the past 10 years. Dendritic cell vaccine and checkpoint blockade strategies were among the first to enter the clinic, with many other strategies such as peptide vaccine, whole cell tumor vaccine, and adoptive T cell transfer (with Chimeric Antigen Receptors) etc. closely following in clinical trials.

Immunophotonics is developing a novel in situ autologous cancer vaccine (InCVAX) by combining thermal laser phototherapy with immunotherapy. InCVAX is a two-step procedure: (1) Delivery of low-power thermal laser to any accessible tumor to cause partial cell death, increase tumor immunogenicity by releasing tumor antigens and Damage Associated Molecular Patterns (DAMPs). This is followed immediately by (2) injection

of our proprietary immunostimulant, N-dihydro-acetylglucosamine (GC), into the laser-treated region to stimulate antigen presenting cells. These two steps work synergistically to enhance the systemic anti-tumor T cell response which is capable of eliminating both primary and metastatic cancers in some patients with advanced, stage III/IV, breast cancer with minimal toxicity. Our approach has the unique benefits of stimulating an immune response against a wide array of tumor antigens, and thus the potential to induce a strong, comprehensive and long-term anti-tumor protection in patients with minimal costs.

Following early data showing efficacy in breast cancer patients, a multi-center, randomized clinical trial is currently underway in South America to consolidate the findings. In addition, we have extended our research of InCVAX to other tumor models and to better understand the mechanism of how GC stimulate the immune system, primarily through activation of antigen presenting cells (APCs). With our data showing therapeutic efficacy of inCVAX in animal and human models, we are confident that inCVAX can bring significant benefit to metastatic cancer patients in the near future.

10065-10, Session 2

Laser photothermal interaction induces immunological effects in laser immunotherapy

Austin Doughty, Ctr. for Interdisciplinary Biomedical Education and Research, Univ. of Central Oklahoma (United States); Shaojie Liu, South China Normal Univ. (China); Feifan Zhou, Ctr. for Interdisciplinary Biomedical Education and Research, Univ. of Central Oklahoma (United States); Hong Liu, The Univ. of Oklahoma (United States); Wei R. Chen, Ctr. for Interdisciplinary Biomedical Education and Research, Univ. of Central Oklahoma (United States)

We have recently developed Laser Immunotherapy (LIT), a targeted cancer treatment modality using synergistic application of near-infrared laser irradiation and in situ immunological stimulation. This study further investigates the principles underlying the immune response to LIT treatment by studying immunological impact of the laser photothermal effect in vivo, in vitro, and ex vivo. Tumor cells were stressed in vitro, and samples were collected to analyze protein expression with a Western Blot. Additionally, a tumor model was designed using bovine liver tissue suspended in agarose gel which was treated using laser interstitially and monitored with both proton-resonance frequency shift MR thermometry and thermocouples. From the bovine liver tumor model, we were able to develop the correlation between tissue temperature elevation and laser power and distance from the fiber tip. Similar data was collected by monitoring the temperature of a metastatic mammary tumor in a rat during laser irradiation. Ultimately, these results show that the laser irradiation of LIT leads to clear immunological effects for an effective combination therapy to treat metastatic cancers.

10065-11, Session 3

Monitoring circulating melanoma cells by in vivo photoacoustic flow cytometry *(Invited Paper)*

Xunbin Wei, Shanghai Jiao Tong Univ. (China)

Melanoma cells have high light absorption due to melanin highly contained in melanoma cells. This property is employed for the detection of circulating melanoma cell by in vivo photoacoustic flow cytometry (PAFC), which is based on photoacoustic effect. PAFC can employ high melanin content of melanoma cells as endogenous biomarkers to detect circulating melanoma cells in vivo. We have developed in vitro experiments to prove the ability of PAFC system of detecting photoacoustic signals from melanoma cells. For in vivo experiments, we have constructed a model of melanoma tumor bearing mice by inoculating highly metastatic murine melanoma cancer cells, B16F10 with subcutaneous injection. PA signals are detected in the blood vessels of mouse ears in vivo. By counting circulating melanoma cells termly, we obtain the number of circulating melanoma cells as melanoma metastasized. Those results show that PAFC is a noninvasive and label-free method to detect melanoma metastases in blood or lymph circulation.

10065-12, Session 3

Dynamical optical imaging monocytes/macrophages migration and activation in contact hypersensitivity *(Invited Paper)*

Zhihong Zhang, Huazhong Univ. of Science and Technology (China)

Inflammatory monocytes/macrophages (Mon/M?) play an important role in cutaneous allergic inflammation. However, their migration and activation in dermatitis and how they accelerate the inflammatory reaction are largely unknown. Optical molecular imaging is the most promising tool for investigating the function and motility of immune cells in vivo. We have developed a multi-scale optical imaging approach to evaluate the spatio-temporal dynamic behavior and properties of immune cells from the whole field of organs to the cellular level at the inflammatory site in delayed type hypersensitivity reaction. Here, we developed some multi-color labeling mouse models based on the endogenous labeling with fluorescent proteins and the exogenous labeling with fluorescent dyes. We investigated the cell movement, cell interaction and function of immunocytes (e.g. Mon/M?, DC, T cells and neutrophils) in the skin allergy inflammation (e.g., contact hypersensitivity) by using intravital microscopy. The long-term imaging data showed that after inflammatory Mon/M? transendothelial migration in dermis, they migrating in interstitial space of dermis. Depletion of blood monocyte with clodronate liposome extremely reduced the inflammatory reaction. Our finding provided further insight into inflammatory Mon/M? mediating the inflammatory cascade through functional migration in allergic contact dermatitis.

10065-13, Session 3

Novel in vivo flow cytometry platform for early prognosis of metastatic activity of circulating tumor cells

Jacqueline Nolan, Chenzhoung Cai, Dmitry A. Nedosekin, Vladimir P. Zharov, Univ. of Arkansas for Medical Sciences (United States)

Approximately 8 million people lose their lives due to cancer each year. Metastatic disease is responsible for ~90% of those cancer-related deaths. Only viable circulating tumor cells (CTCs) that can survive in the blood circulation can create secondary tumors. Thus, real-time enumeration of CTCs and assessment of their viability in vivo has great biological significance. However, little progress has been made in this field. Conventional flow cytometry is the current technique being used for the assessment of cell viability, but there are many limitations to this technique: 1) cell properties may be altered during the extraction and processing method; 2) collection of cells from blood prevents the long-term study of individual cells in their natural biological environment; and 3) there are time-consuming preparation procedures. Whether it be for the assessment of antitumor drugs, where induction of apoptosis or necrosis is the preferred event, or the identification of nanoparticle-induced toxicity during nanotherapeutic treatment, it is clear that new approaches for assessment of the viability circulating blood cells and CTCs are urgently needed. We have developed a novel high speed, multicolor in vivo flow cytometry (FC) platform that integrates photoacoustic (PA) and fluorescence FC (PAFFC) and demonstrate its ability to enumerate rare circulating normal and abnormal (e.g. tumor) cells and assess their viability (e.g. apoptotic and necrotic) in a mouse model.

10065-14, Session 3

Observation of the immune response of cells and tissue through multimodal label-free microscopy

Nicolas Pavillon, Nicholas I. Smith, Osaka Univ. (Japan)

The observation of the immune response often requires the use of labels targeting for example specific intracellular or membrane proteins in fluorescence microscopy or flow cytometry systems, or secreted cytokines through enzyme-linked immunosorbent assays (ELISA). We present applications of a label-free approach to assess the immune response based on the combination of interferometric microscopy and Raman spectroscopy, which makes it possible to simultaneously acquire morphological parameters and molecular information about the intracellular content.

In particular, we show how this multimodal approach can be employed to assess the activation state of macrophage cells. The full-field imaging approach of quantitative phase microscopy enables a high-throughput acquisition of the morphological parameters, making it possible to derive a statistical model for predicting the activation state of macrophages under an in vitro model of bacterial infection at single-cell level.

Furthermore, the Raman measurements performed in parallel enable the assessment of the molecular changes occurring upon activation. The accuracy of the model is assessed through ELISA measurements and detection of reactive oxygen species, known to be secreted upon activation.

We also present an application for three-dimensional imaging of tissue, where the interferometric microscopy is coupled to coherence gating for depth measurements, and coupled with Raman spectroscopy. In this case, it is possible to rapidly identify tissue structures of interest through the wide-field interferometric channel, which can be targeted with the Raman system to assess the local molecular content to further assess immunological responses.

10065-15, Session 3

Hyperspectral microscopy and its biomedical applications in cancer diagnosis

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Hyperspectral microscopy (HSM) is an emerging spectral imaging modality that combines the advantages of both microscopy and spectroscopy in one device. Hyperspectral imaging was originally used for remote sensing, however it has recently become a powerful process analytical tool for non-destructive medical imaging and diagnostics with the advantage of acquiring two-dimensional images across a wide range of spectrum. During the progression of disease, the absorption, fluorescence, and scattering characteristics of tissue may change, which contains important structural, biochemical or physiological information. Therefore, the reflected, fluorescent, and transmitted light from tissue captured by HSM carries quantitative diagnostic information about tissue pathology. In this paper, we present a hyperspectral microscopy system to quantitatively measure and image the normal and cancer tissues with high spectral and spatial resolutions. The approach proposed here may have potential biomedical applications in early disease detection and diagnosis.

10065-16, Session 3

A lipid-based nano-regulator for cancer immunotherapy

Yuan Qian, Sha Qiao, Zhihong Zhang, Huazhong Univ. of Science and Technology (China)

In the application of nanotechnology in cancer immunotherapy, antigen presenting cells (APCs, dendritic cells and macrophages) are preferable target due to their endocytic capacity and suppressed phenotype. Recently, we developed a lipid-based core-shell nanocarrier, which is stabilized by changeable fusion peptides and possesses a sub-30 diameter. With the different peptides, the nanoparticles (NPs) could either target to dendritic cells (DCs) in lymph nodes (LNs) or tumor associated macrophages (TAMs) in tumor environment. After subcutaneous injection, the NPs could targeted deliver the encapsulated antigen peptides (APs) and adjuvants (CpG-ODN) to dendritic cells in LNs, and lead to the antigen presenting and activation of cytotoxic T lymphocytes against tumor. In other case, after systemic administration, the immune regulatory molecules were carried by NPs and targeting delivered to specific immunocytes in tumor microenvironment resulting in the immunosuppressive state broken and tumor growth inhibition.

10065-17, Session 4

Fluorescent imaging and artificial neural networks in the classification of vascular immune reaction *(Invited Paper)*

Vyacheslav Kalchenko, Ilya Kuznetsov, Guillaume Molodij, Yuri Kuznetsov, Weizmann Institute of Science (Israel); Igor Meglinski, Univ. of Oulu (Finland); Alon Harmelin, Weizmann Institute of Science (Israel)

We developed a simple approach dedicated for characterization of immune responses during preclinical studies. The main principle of the proposed methodology is based on the use of fluorescence imaging of vascular permeability in response to topical administration of immunologically active agents (IAA).

In previous studies we proposed a quantitative analysis based on methods derived from astronomical observations, in particular by using a space-time Fourier filtering analysis followed by a polynomial orthogonal modes decomposition.

Herein we demonstrate the usability of a new methodology for quantification, classification and automatic analysis of immune responses to IAA. The proposed methodology is a combination of supervised and unsupervised techniques based on Artificial Neural Networks (ANNs) as one of deep learning method.

We also specifically focus on the application of Convolutional Neural Network (CNN) in which the connectivity pattern between its neurons is inspired by the organization of the animal visual cortex. Classification using CNN differ from other methods – through learning process neural network find regularity in data and store different abstraction levels (features) inside its layers.

Application of CNN in quantitative analysis of vascular permeability during immune reaction allow us to allocate not only time features of immune reaction, but also take into account structure of the vascular network and other tissue structures.

We also summarize advantages and disadvantages of the use of various types of ANNs and deep learning during optical imaging of immune responses.

10065-18, Session 4

Improving efficacy of metastatic tumor segmentation to facilitate early prediction of ovarian cancer patients' response to chemotherapy *(Invited Paper)*

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University of Oklahoma Health Sciences Center (United States); Samuel Cheng, Hong Liu, Bin Zheng, Yuchen Qiu, The Univ. of Oklahoma (United States)

Accurate tumor segmentation is a critical step in the development of the computer-aided detection (CAD) based quantitative image analysis scheme for predicting the early chemotherapy response of ovarian cancer patients. The purpose of this investigation is to assess the efficacy of several different methods to segment the metastatic tumors occurred in different organs of ovarian cancer patients. In this study, we used segmentation scheme consisting of eight different algorithms, which can be divided into three groups: 1) Region growth based methods; 2) Canny operator based methods; and 3) Partial differential equation (PDE) based methods. A number of 138 tumors acquired from 30 ovarian cancer patients were used to test the performance of these eight segmentation algorithms. The results demonstrate that the PDE based algorithm achieved a higher performance than the traditional region growing and Canny boundary operator based algorithms. Furthermore, each of the tested tumors can be successfully segmented by at least one of the eight algorithms without the manual boundary correction. This study may provide meaningful reference for developing CAD-based quantitative image feature analysis scheme to more accurately predict the early response of ovarian cancer patients to the chemotherapy in the future clinical trials or practice.

10065-20, Session 4

Detectability comparison of simulated objects within a dense breast phantom using high energy phase sensitive x-ray imaging and conventional imaging systems

Muhammad U. Ghani, Molly D. Wong, Di Wu, Bin Zheng, The Univ. of Oklahoma (United States); Wei R. Chen, Univ. of Central Oklahoma (United States); Laurie L Fajardo, Department of Radiology and Imaging Sciences,University of Utah (United States); Xizeng Wu, The Univ. of Alabama at Birmingham (United States); Hong Liu, The Univ. of Oklahoma (United States)

The objective of this study was to demonstrate the detectability of simulated objects within a dense breast phantom using high energy x-rays for phase sensitive breast imaging in comparison with conventional imaging. A 5 cm thick contrast-detail (CD) phantom representing a compressed breast consisting of 70% glandular and 30% adipose tissue ratio by weight was used. The phantom has a 6 ? 6 matrix of holes with milled depths ranging from 1 to 0.1 mm and diameters ranging from 4.25 to 0.25 mm representing the simulated tumors. All the CD phantom images were acquired using a micro-focus x-ray source with a 50 μm focal spot and a flat panel detector a 50 μm pixel pitch. Phase sensitive images were acquired at 120 kVp, 4.5 mAs with source to object distance (SOD) of 68 cm and a magnification factor (M) of 2.5. Conventional images were acquired at 40 kVp, 12.5 mAs and 120 kVp, 4.5 mAs with a source to imaging distance (SID) of 68 cm. The observer study and contrast-to-noise ratio (CNR) indicates an improvement by the phase sensitive images as compared to the conventional images. The edge enhancement provided by the phase sensitive images warrants in identifying boundaries of malignant tissues and in providing optimal results in phase retrieval process. The potential demonstrated by this study for imaging a dense breast with a high energy phase sensitive x-ray imaging to improve tumor detection in warrants further investigation of this technique.

10065-21, Session 4

Characteristic performance investigation of a photon counting detector for x-ray fluorescence imaging applications

Liqiang Ren, The Univ. of Oklahoma (United States) and Univ. of Central Oklahoma (United States); Di Wu, Yuhua Li, The Univ. of Oklahoma (United States); Wei R. Chen, Univ. of Central Oklahoma (United States); Bin Zheng, Hong Liu, The Univ. of Oklahoma (United States)

The characteristic performance of a photon counting detector for X-ray fluorescence (XRF) imaging of gold nanoparticles (GNPs) is investigated. The investigations are first performed in three aspects: X-ray photon energy (keV) to pulse height (mV) conversion, noise floor determination, and linear detection ranges. Then, theoretical models are applied to evaluate the detection efficiency of X-ray photons with respect to an increased incident photon rate. Last, through exciting 100% pure GNPs by a conventional X-ray tube operated at a voltage of 110kVp, we acquire XRF spectrum in the threshold mode, based on which multi-energy thresholds are selected for XRF imaging of GNPs with low concentrations. Preliminary XRF imaging results of GNPs obtained in the imaging mode are presented and analyzed. This investigation study is essential to the development of fast and accurate XRF imaging of GNPs as well as other high atomic (Z) imaging contrast agents absorbed in cancerous cells.

10065-22, Session 4

Measurements of gold nanoparticle concentration with k-shell x-ray fluorescence spectrum

Di Wu, Yunhua Li, Molly D. Wong, Muhammad U. Ghani, Bin Zheng, The Univ. of Oklahoma (United States); Wei R. Chen, Univ. of Central Oklahoma (United States); Hong Liu, The Univ. of Oklahoma (United States)

X-ray fluorescence imaging technique by using gold nanoparticles was demonstrated potentially improves diagnosis imaging specificity by targeting nanoparticles to tumor areas. The x-ray fluorescence detectability of low-concentration GNPs distribution determines the detecting ability and sensitivity of this technique. In this study, we are going to measure the GNP k-shell fluorescence by using a 100 mm long scatter-eliminating collimator to further improve the detecting sensitivity compare to literatures. The GNPs were suspended in deionized water to acquire different concentrations. The GNP suspensions were excited by a focal spot x-ray tube with 130 kVp, 300 μA x-ray exposures. The emissions of the GNP fluorescence were measured by a spectrometer located with an angle of 90 degree as respect to the excitation beam. The fluorescence acquisition durations for each concentration mode were 3000 s. A 1.0 mm Pb filter and a 1 mm Al filter were utilized to optimize excitation beam and fluorescence emission. As a result, the k-shell fluorescence peaks, 66.99 keV and 68.80 keV, were measured and observed in 0.1, 0.2, 0.4, 0.8, 1.0, 2.0 and 4.0 mg/mL concentration modes (0.01 %, 0.02 %, 0.04 %, 0.08 %, 0.10 %, 0.20 % and 0.40 % in weight concentration, respectively). The resultant calibration curves performed linear relations between the GNP suspension concentrations and the number of photons of the fluorescence peaks. Therefore, the detection sensitivity of GNP fluorescence was successfully improved by an order of magnitude and observably reached 0.1 mg/mL (0.01 % in weight concentration).

10065-23, Session PMon

Artesunate induces ROS-dependent apoptosis via a Bax-mediated intrinsic pathway in Huh-7 and Hep3B cells

Yilin Pang, Tongsheng Chen, South China Normal Univ. (China)

Artesunate (ARS), a semi-synthetic derivative of artemisinin (ART), has been demonstrated to possess antitumor activity in various human tumor cells. ARS induces apoptosis in diverse human cancer cell lines. Although reactive oxygen species (ROS) is responsible for the antimalarial activity of ARS, the action of ROS in ARS-induced apoptosis is controversial. This study aims to investigate the molecular mechanism by which ARS induces apoptosis in human hepatocellular carcinoma cells (Huh-7 and Hep3B cells). ARS effectively induced externalization of phosphatidylserine (PS), depolarization of mitochondrial membrane, release of cytochrome c from mitochondria, and activation of caspase-9 and 3, characteristics of the intrinsic apoptosis. Pretreatment with antioxidant N-Acetyle-Cysteine (NAC) completely blocked ARS-induced reactive oxygen species (ROS) generation and apoptosis in the two cell lines. ARS increased cellular iron ions level in Huh-7/Hep3B cells, but decreased cellular iron ions level in HepG2 cells. Pifithrin-alpha (PFT), an inhibitor of p53, significantly enhanced ARS-induced cytotoxicity in HepG2 cells, and the forced expression of wild-type p53 significantly enhanced ARS-induced cytotoxicity in Hep3B cells. In addition, ARS induced translocation and activation of the proapoptotic Bax, and silencing Bax remarkably inhibited ARS-induced apoptosis and ??m collapse in Huh-7 and Hep3B cells, demonstrating the key role of Bax in ARS-induced apoptosis. Collectively, our data demonstrate that ARS induces ROS-dependent apoptosis via a Bax-mediated intrinsic pathway in Huh-7 and Hep3B cells.

10065-24, Session PMon

Role of peroxynitrite in SNP-induced apoptosis of HepG2 cells

Yingyao Quan, Xiao-Ping Wang, The First Affiliated Hospital of Jinan Univ. (China)

Sodium nitroprusside (SNP) has been widely used as an exogenous nitric oxide (NO) donor to explore the NO-mediated biological effects in human hepatocellular carcinoma cells (HepG2 cells). We have recently found that NO-independent Fenton reaction play a key role in SNP -induced rabbit chondrocytes apoptosis. This study aims to investigate the reliable mediators that mediate the SNP-induced cytotoxicity in HepG2 cells. Data shows that SNP induced HepG2 death in apoptotic fashion. SNP induced a marked increase in NO, reactive oxygen species (ROS), superoxide anion (O2•?), peroxynitrite (ONOO-), hydrogen peroxide (H2O2) and iron ions level. NO scavenger (PTIO), O2•? scavenger (SOD), ONOO- scavenger (FeTPPs) and iron ions chelator (DFO) significantly inhibited SNP-induced decrease in cell viability, respectively. In contrast, ROS scavenger (NAC) promoted SNP-induced cytotoxicity and H2O2 scavenger (CAT) had no protective effects on SNP-induced cytotoxicity. FeTPPs pretreatment remarkably prevented SNP-induced HepG2 apoptosis, decrease of ??m and caspases activation. In addition, NO donor (NOC-5) and ONOO- donor (SIN-1) also induced significant cytotoxicity in HepG2 cells. Collectively, our data demonstrate that the peroxynitrite plays a key role in SNP-induced apoptosis in HepG2 cells.

10065-25, Session PMon

Clinical trials for metastatic cancers using inCVAX: Progress and milestones of a biotech company

Tomas Hode, Luciano Alleruzzo, Joseph Raker, Siu Kit Lam, Immunophotonics, Inc. (United States); Robert E. Nordquist, Wound Healing of Oklahoma, Inc. (United States); Feifan Zhou, Wei R. Chen, Ctr. for Interdisciplinary Biomedical Education and Research, Univ. of Central Oklahoma (United States)

A novel drug, N-dihydro-galacto-chitosan (GC), is being developed by Immunophotonics Inc. for use in an in situ autologous whole-cell cancer vaccine (inCVAX) against metastatic cancers. inCVAX combines phototherapy and immune activation by GC to induce systemic anti-tumor T-cell responses in the hosts. Immunophotonics and its academic partners have spent many years conducting nonclinical research, developing CMC and conducting initial clinical research. In 2016 the company initiated an expansion of its previous first-in-human clinical trial in South America for advanced breast cancer patients. The process of developing the inCVAX approach from a laboratory setting and into clinical trials requires significant efforts from a group of dedicated engineers, scientists, physicians, investors, and business professionals. This talk will chronicle the progress and milestones of the scientific achievement, medical progress, and business development of Immunophotonics.

10065-26, Session PMon

Development of ex vivo model for determining temperature distribution in tumor tissue during photothermal therapy

Shaojie Liu, South China Normal Univ. (China) and Univ. of Central Oklahoma (United States); Austin Doughty, Feifan Zhou, Univ. of Central Oklahoma (United States); Hong Liu, The Univ. of Oklahoma (United States); Wei R. Chen, Univ. of Central Oklahoma (United States)

Temperature distribution in tissue is a crucial factor in determining the outcome of photothermal therapy in cancer treatment. In order to determine the temperature distribution in tumor tissue during laser irradiation, we developed a novel ex vivo device to simulate the photothermal therapy on tumors. A 35°C trermostatic incubator was used to provide an environment with body temperature. Different biological tissues (chicken breast, bovine liver, rat tumor) buried inside gel were considered to simulate tumor tissue. An 805-nm laser was used to irradiate target tissue. Different power settings (from 0.5 to 3 W) were applied with different irradiation durations (10, 15 and 20min). An optical fiber with an interstitial cylindrical diffuser (10 mm) was directly inserted in the center of tissue and the needle probes of a thermocouple were inserted in the tissue parallel to the laser fiber at different distances with a separation of 1.28 mm between each needle probe. All the procedures were performed in the incubator. Under the interstitial laser irradiation of 3W, the target tissue experienced a steady temperature increase over a period of 75s, 100s and 20s for rat tumor, bovine liver and chicken breast, respectively. The tissue temperature reached a plateau of 47°C, 48°C and 10°C for rat tumor, bovine liver, and chicken breast, respectively. Therefore, bovine liver tissue buried inside the gel with water-keeping film in the incubator appeared to be a proper model to simulate animal tumors. Our work provides an ex vivo model for determining temperature distribution during tumor interstitial photothermal therapy.

10065-27, Session PMon

Comparing the photothermal effects of gold-nanorods and single-walled carbon nanotubes in cancer studies

Connor L. West, Aamr M. Hasanjee, Roman F. Wolf II, Kegan Silk, Feifan Zhou, Univ. of Central Oklahoma (United States); Hong Liu, The Univ. of Oklahoma (United States); Wei R. Chen, Univ. of Central Oklahoma (United States)

Laser Immunotherapy (LIT) is an innovative cancer treatment modality that is aimed specifically to treat late-stage, metastatic cancer treatment. LIT uses laser irradiation and strong immune system stimulation to produce a systemic anti-tumor immune response against metastatic cancer. In recent years, nanoparticles have been used to strengthen the photothermal effect of the laser irradiation by absorbing laser light energy and converting it into thermal energy. Formerly, single-walled carbon nanotubes (SWNTs) have been the preferred nanoparticle, but recent studies have shown that gold nanorods (AuNRs) can have a similar photothermal effect to the SWNTs. However, AuNRs are thought to be a safer alternative to SWNTs, due to the precedence of gold products being used in various other treatments for humans. The goal of this study is to directly compare the photothermal effects of AuNRs to SWNTs. Both nanoparticles were tested for photothermal efficacy under the irradiation of the same near-infrared laser, using gel phantom tumor models, aqueous solutions, and metastatic cancer cell cultures. We observed that the AuNRs were equally or more effective than SWNTs in absorbing the laser light and converting it into thermal energy. In solution and gel studies, AuNRs proved to be more thermally efficient than SWNTs, while in cell studies, AuNRs and SWNTs appeared to have the same thermal effect, but further studies are needed to assess the cytotoxicity of both nanoparticles. Given these results, AuNRs are comparable to SWNTs, and better in some aspects, thus paving the way for AuNRs to replace SWNTs in LIT treatments. These results will contribute to our future studies in the development of nanoparticle-enhanced photothermal therapy for cancer treatment.

10065-28, Session PMon

The mBeRFP Is suitable for multi-color intravital multi-photon imaging

Zhihong Zhang, Fei Yang, Huazhong Univ. of Science and Technology (China)

Multi-color intravital multi-photon imaging technology requires that fluorophores of different emission peaks should be excited with a single infrared laser. The mKate-derived Large Stokes' Shift (LSS) red fluorescent protein, mBeRFP, is a bright genetic reporter for multi-color multi-photon imaging. It has a maximum excitation at 446 nm and a maximum emission at 615 nm, making it suitable for simultaneous excitation with cyan fluorescent proteins. Herein, we transfected mBeRFP gene into murine melanoma B16 with a PiggyBac system and screened for a stable cell line, mBeRFP-B16. Within a murine dorsal window chamber model, we visualized mBeRFP-B16 tumor in vivo with two-photon excitation throughout 770 - 960 nm. Finally, we realized a four-color imaging in vivo, including mCerulean, mBeRFP, Venus and SHG, with two-photon excitation at 920 nm, proving mBeRFP a useful tool for multi-color intravital imaging reporter.

10065-29, Session PMon

Anti-hepatocarcinoma effects of resveratrol nanoethosomes against human HepG2 cells

Xiangping Meng, Medical Technology and Engineering College, Henan University of Science and Technology (China); Zhen Zhang, Guangdong Pharmaceutical Univ. (China); Tongsheng Chen, South China Normal Univ. (China); Yifei Wang, Institute of Biological Medicine, Jinan Univ. (China); Zhi-ping Wang, Guangdong Pharmaceutical Univ. (China)

Hepatocarcinoma, a malignant cancer, threaten human life badly. It is a current issue to seek the effective natural remedy from plant to treat cancer due to the resistance of the advanced hepatocarcinoma to chemotherapy. Resveratrol (Res) has been widely investigated with its strong anti-tumor activity. However, its low oral bioavailability restricts its wide application. In this study, we prepared resveratrol nanoethosomes (ResN) via pH-gradient method. The in vitro anti-hepatocarcinoma effects of ResN relative to efficacy of bulk Res were evaluated on proliferation and apoptosis of human HepG2 cells. ResN were spherical vesicles and its particle diameter, zeta potential were (127.6 ± 1.8) nm and (-9.4 ± 0.3) mV, respectively. ResN exhibited significant inhibitory effects against human HepG2 cells by MTT assay, and the IC50 value was 6.50 ?g /ml (11.32 ?g /ml of Res bulk solution). By flow cytometry assay, the apoptosis rates of HepG2 cells induced by ResN increased?and there was an increase in S phase cells. The results demonstrated ResN could effectively inhibit and induce the apoptosis of HepG2 cells, which can also prolong the phase of inhibitory effect of Res against HepG2 cells.

10065-30, Session PMon

Evaluation of anti-hepatocarcinoma capacity of puerarin nanosuspensions against human HepG2 cells

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Resveratrol (Res, trans-3,4,5-trihydroxystilbene) is a kind of phytoalexin produced by plant which can stimulate the natural plant defense function. It can also affect a wide range of organs and tissues of animal and human to bring beneficial effects, including the prevention of abnormal platelet aggregation, antopxidation, anti-cancer and anti-aging potential, but its anti-inflammatory mechanism is unclear. In this research, we measured the effect of Res on the biochemical indexes of macrophages, to investigate whether resveratrol affected the biochemical index of macrophages under physiological conditions. IL-1 family, A class of cytokines produced by a variety of cells and acting on a variety of cells which can promote T helper type 2 (Th2)-associated inflammations and allergic diseases. LPS-induced macrophage activation was investigated, Res can counteracted LPS-induced lipoperoxidation (0.122 ?g/ml) and decreased superoxide dismutase (SOD) activity (0.053 ?g/ml), more importantly, Res can reduce the inflammatory mediators IL-1 family (0.024?g/ml) and Th2 secretion of macrophage (0.052?g/ml). All these data suggest that Res is capable of alleviating LPS-induced inflammation , and that its mode of action may involve Antioxidant capacity.

10065-31, Session PMon

The effects of prolonged oral administration of gold nanoparticles on the morphology of hematopoietic organs

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Currently, the usage of gold nanoparticles as photosensitizers and immunomodulators for plasmonic photothermal therapy has attracted a great attention of researches and end-users. In our work the influence of prolonged oral administration of gold nanoparticles (GNs) with different sizes on the morphological changes of hematopoietic organs was investigated. The 24 white outbred male rats weighing 180-220 g were randomly divided into groups and administered orally for 30 days the suspension of gold nanospheres with sizes 2, 15 and 50 nm at a dosage of 190 μg/kg of animal body weight. To prevent nanoparticles aggregation in a tissue and enhance biocompatibility, GNs were functionalized with thiolated polyethylene glycol. The withdrawal of the animals from the experiment and sampling of spleen, lymph nodes and bone marrow tissues for morphological study were performed a day after the last administration. In the spleen the boundary between the red and white pulp was not clearly differ in all experimental groups, lymphoid follicles were significantly increased in size, containing bright germinative centers represented by large blast cells. The stimulation of lymphocyte and myelocytic series of hematopoiesis was recorded at morphological study of the bone marrow. The number of immunoblasts and large lymphocytes was increased in all structural zones of lymph nodes. The more pronounced changes were found in the group with administration of 15 nm nanoparticles. Thus, the morphological changes of cellular components of hematopoietic organs have size-dependent character and indicate the activation of the migration, proliferation and differentiation of immune cells after prolonged oral administration of GNs.

10065-32, Session PMon

Using FRET to quantify changes in integrin structures in human leukocytes induced by chemoattractants with multifrequency flow cytometry

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Time-resolved flow cytometry is a unique method that involves the detection of the average fluorescence lifetime as a cytometric parameter to report subtle changes in cell morphology, cell phenotype, and microenvironmental changes. Measuring average fluorescence lifetime is helpful when discriminating between more than one emission signal from a single cell because of spectrally overlapping emission. In this contribution, we present preliminary measurements toward a study that advances simple time-resolved flow cytometry and introduce a technique to measure multiple fluorescence lifetimes. We use two approaches: (1) phasor plot analyses, and (2) a square-wave modulation approach to increase the number of phase-shifts measured with cytometry and collect multiple fluorescence lifetime value per cell from single cells labeled with a FRET pair. Specifically, donor fluorophore FITC fluorescence lifetime is measured with regards to its proximity to the acceptor fluorophore. We hypothesize multiple-lifetime time-resolved flow cytometry approach can resolve changes in FRET in order to study integrin structures on the surface of leukocyte cells. Our results show that FITC has an average lifetime of 4.2 +/- 0.1 nsec, and an average fluorescence lifetime of 2.4 nsec +/-0.2 nsec when engaged in FRET. Diminishing FRET average fluorescence lifetime of FITC was measured a 3.1 +/- 0.5 nsec. Our results include phasor plots showing wide distributions of fluorescence lifetimes on a per cell basis, suggesting the existence of multiple fluorescence lifetimes. Our square wave multi-frequency results show multiple fluorescence lifetimes that favor longer FITC fluorescence lifetimes. The impact of this work demonstrates a mean to add quantitative information to a FRET efficiency value when multiple fluorescence lifetime is measured per cell. The implication is that there is more than one integrin conformation occurring within a cell population.

10065-33, Session PMon

Predicted immune dynamics during laser immunotherapy

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We extend our model of the antitumor immune response initiated by laser-immunotherapy treatment to more closely examine three key steps in the immune response: 1) tumor antigen acquisition by antigen-presenting dendritic cells, 2) cytotoxic T cell (CTL) priming by lymphatic dendritic cells, and 3) tumor cell killing by activated CTLs. In particular, we explore the sensitivity of dynamics to assumptions about the mathematical form of the interactions (i.e., mass-action versus non mass-action contact and encounter rates). We make comparisons of predicted dynamics to available animal model data, when available, and give implication for treatment outcomes.

10065-34, Session PMon

Inactivation pathogenic microorganisms in water by laser methods

Alexey Iakovlev, St. Petersburg State Forest Technical Univ. (Russian Federation); Aleksandr S. Grishkanich, Sergey V. Kascheev, Julia Ruzankina, ITMO Univ. (Russian Federation); Igor S. Sidorov, Univ. of Eastern Finland (Finland)

In developing countries today, the poor quality of water is responsible for about 20% of all diseases. If current patterns of water use problem of drinking water will soon acquire a global character. So now the problem of drinking water quality has not lost its relevance. With the emergence of pathogens such as typhoid, cholera, anthrax, staphylococcus, E. coli waterborne and degrade the quality of drinking water and cause disease. Proposed compact laser device of water treatment. As a result of the research the following methods have been proposed for controlling harmful microorganisms: sterilization of water by laser radiation at wavelengths of 425 nm, 355 nm and 308 nm. The results of theoretical and experimental studies on the development and establishment of a system of ultraviolet disinfection of water for injection (UFOVI) intended for research sterilized water for injections. The pipe created a strong turbulent water flow. Performance irradiation laminar flow of 1.5 liters per second. Irradiation was carried out at three wavelengths 425 nm, 355 nm and 308 nm with energies semiconductor laser diode arrays to 4 MJ / cm3. Wavelength tuning implemented current in the range of 10 nm. For large capacities, we have developed a miniature solid state laser, which was used in fluid microorganisms inactivator. In the water treatment process breaks up to 98% of microbes, but can be left among pathogenic viruses destruction which requires special handling.

10065-35, Session PMon

Effects of glycated chitosan on metastatic cancer cell migration

Elivia Layton, Aamr M. Hasanjee, Rachel McNamar, Blake Young, Alex Pettitt, Feifan Zhou, Melville B. Vaughan, Wei R. Chen, Univ. of Central Oklahoma (United States)

The most promising treatment method for metastatic cancer would destruct the primary tumor, and eradicate any metastasis, as well as build a long-term immunity to the disease. One possible solution to this problem is laser immunotherapy (LIT). LIT combines the application of a photothermal laser with an immunoadjuvent such as Glycated Chitosan (GC). Studies using GC have been very successful in combination with single-walled carbon nanotubes (SWNTs), as well as irradiation of near-infrared laser. It would be crucial to determine if GC alone can inhibit the migration of metastatic cancer cells. The migration of 4T1 cells treated with GC was evaluated and compared to those under similar conditions with no treatment, as well as those treated with SWNT, and a combination of SWNT and GC. The methods used to evaluate this movement involved a scratch-well migration assay and a 3D collagen matrix. The 4T1 cells treated with GC moved only approximately half the distance of those untreated. The migration properties of cells cultured in collagen matrices are inhibited by SWNT-GC. Nested matrices were used to determine whether GC and SWNT-GC inhibit migration. Matrices were injected with cells to simulate a tumor in vivo, and migration were observed using a microscope. The collagen matrices were then be irradiated by the laser to complete the simulation of LIT treatment. Previous data suggest that the LIT treatment would inhibit migration and cause apoptosis. This is very encouraging news because it could indicate a possibility for more comfortable means of treatment. These results could further explain why LIT in combination with an immunoadjuvant such as GC has gained popularity in cancer research.

10065-36, Session PMon

A light therapy for treating Alzheimer's disease

Xunbin Wei, Shanghai Jiao Tong Univ. (China)

It is generally believed that there are some connections between Alzheimer's disease (AD) and amyloid protein plaques in the brain. The typical symptoms of AD are memory loss, language disorders, mood swings and behavioral issues. Currently, the main therapeutic method is pharmacotherapy, which may temporarily reduce symptoms, but has many side effects. Infrared (IR) light therapy has been studied in a range of single and multiple irradiation protocols in previous studies and was found beneficial for neuropathology. In our research, we have studied the effect of infrared light on AD through mouse model. We designed an experimental apparatus for treating mice, which primarily includes a therapeutic box and a LED array, which emits infrared light. After the treatment, we assessed the effects of infrared light by testing cognitive performance of the mice in Morris water maze. Our results show that infra-red therapy is able to improve cognitive performance in the mouse model. It might provide a novel and safe way to treat Alzheimer's disease.

10065-37, Session PMon

Successful treatment of cutaneous granuloma caused by candida guilliermondii using in situ photoimmunotherapy

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Background and Purpose. Cutaneous granulomas caused by Candida guilliermondii are difficult to cure. In situ photoimmunotherapy (ISPI) is a novel method composed of local photothermal therapy and immunoadjuvant. In this study, ISPI was used the first time clinically for cutaneous granuloma caused by itraconazole-resistant C.guilliermondii.

Case Description. A 10-week cycle of ISPI was composed of (1) 5% imiquimod applied topically every other day and (2) irradiation of lesions with an 808-nm diode laser at Days 14, 28, 42, and 56. A patient with cutaneous granuloma caused by itraconazole- and ALA-PDT-resistant C. guilliermondii was treated with ISPI.

Outcomes. A thermal gradient on the lesion surface was generated and the highest temperature reached 50.0?. After 4 cycles of ISPI treatment, the lesions were eliminated without recurrence during a 12-month follow-up.

Discussion. Our results demonstrate that ISPI can be used as an effective treatment modality for cutaneous fungal granuloma.

10065-38, Session PMon

Glycated chitosan combined radiation induced sustained DNA damage response in murine breast cancer cells

Chun-Yuan Chang, Chun-Yu Wang, National Yang-Ming Univ. (Taiwan); Chung-Yih Wang, Cheng Hsin General Hospital (Taiwan); Yi-Jang Lee, National Yang-Ming Univ. (Taiwan)

Background

Glycated chitosan (GC) is a novel immunoadjuvant that can facilitate various primary therapeutic modalities. Little is known if radiotherapy would be enhanced by GC. In this study, we would like to investigate whether GC will influence the radiation responses using the murine breast cancer cells.

Methods

4T1 murine breast cancer cell and liver metastatic 4T1-L cells were used for GC treatment followed ?-rays irradiation. DNA damage was evaluated using the single cell electrophoresis (comet assay). The DNA damage related molecule was examined using Western blot analysis. Cell cycle distribution and apoptosis were examined using the flow cytometry.

Results

The current results showed that GC could enhance the radiosensitivity of 4T1 cells and 4T1-L cells at low dose and high dose of gamma?-rays, respectively. The tail moment obtained from comet assay showed that GC tended to loose the chromatins. The DNA damage level was sustained in GC combined radiation compared to radiation alone after 24 hours of treatment. The expression of gamma?-H2AX was also increased by GC treated cells. However, GC did not affect the cell cycle distribution, although the sub-G1 population was increased by combined treatment.

Conclusion

GC may pre-render the chromatin structural change that enhances the DNA damage caused by gamma?-rays.

10065-39, Session PMon

Cytocompatibility and immunomodulatory properties of the NaYF4-based upconversion nanoparticles

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Shaozhuang Yang, Wei Yan, Ming Zhu, Jun Song, Tymish Y. Ohulchanskyy, Junle Qu, Shenzhen Univ. (China)

The upconversion nanoparticles (UCNPs) have been proved to contain excellent optical properties, leading to increasing biological and medical applications, including bio-imaging and photo-therapy. To explore their potential applications, it is essential to have a better understanding of the cytotoxicity and biocompatibility of UCNPs, especially their immunomodulatory effects. Here, a series of ~30 nm NaYbF4:Er-based core-shell-shell UCNPs, in which size effect could be harnessed to enhance 654 nm emission, were synthesized using Ostwald-Ripening strategy. The surfaces of resulting UCNPs were then modified with polyacrylic acid (PAA). Both the bare UCNPs and the PAA-modified UCNPs were applied onto the mouse macrophage Raw264.7 cells for a series of biological tests to investigate their potential cytotoxicity, including cell viability assay and apoptosis test. Meanwhile, the secretion of TNF-? and IL-6 by Raw264.7 cells induced by the UCNPs were measured to analyze their immunomodulatory effects. Taken together, these results showed that both the bare and the PAA-modified UCNPs have low cytotoxicity and stimulatory effects on immune cells, indicating their promising biological applications.

10065-40, Session PMon

Use of single-walled carbon nanotubes to inhibit metastatic cancer cell motility

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Non-invasive laser immunotherapy (NLIT) is being developed as a treatment method for metastatic cancer which can directly destroy primary tumors and induce effective systemic anti-tumor responses. This is achieved by synergistically combining the photothermal and immunological effects of non-invasive laser irradiation and immunologically-modified nanostructures. Generally, NLIT utilizes single-walled carbon nanotubes bound (SWNT) to glycated chitosan (GC) to form (SWNT-GC), a photosensitizing and immune system-boosting nanostructure. Although NLIT's ability to induce anti-tumor and anti-metastatic immune response has been demonstrated, the immunological mechanism by which this is accomplished is unknown. Thus, this study aims to determine SWNT-GC's role in the anti-migratory effects of NLIT by exploring the anti-migratory effects of SWNT. SWNT's anti-migratory effects were assessed using various metastatic cancer cell types, including 4T1, Panc-02-H7, and DMBA-4. Wound healing assays and scratch assays were used to compare inhibition of cell motility and invasion induced by SWNT and GC separately. Results of previous studies regarding GC's effects on cancer cell motility were replicated and showed that GC significantly inhibits breast cancer cell motility. Similarly, SWNT are also capable of reducing cancer cell motility. Furthermore, anti-migratory effects of 980-nm laser irradiation paired with SWNT and GC on various cancer cell lines are being investigated. The results of this study shed light on the mechanism by which NLIT inhibits metastases and promotes anti-tumor immune responses.

10065-41, Session PMon

Study on light triggered gene therapy for EML4-ALK fusion mutant NSCLC by nanoparticle drug carrier system based on gold nanoshell

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The clinical experiments about EML4-ALK fusion mutant non-small cell lung cancer revealed that the fusion gene of EML4-ALK could not coexist with the mutation such as EGFR?KRAS, which leads to a lack of effective therapies of EML4-ALK fusion mutant non-small cell lung cancer. The early study of our research group showed that the inhibition of ALK could promote the expression of the cancer suppressor gene BIM, which is in the cell of EML4-ALK fusion mutant non-small cell lung cancer and could lead to apoptosis. This study plans to put the interference sequences of ALK in the gold nanoshell carrier, considering the traits of gold nanoshell like high drug loading capacity, tumor targeting and controllable light triggered drug release etc. And a controllable nano drug loading carrier system, Au@ shell-siRNA, is built. By studying the properties of this system, the synergy therapy containing hyperthermia, gene target therapy in vivo and in vitro which aims to EML4-ALK fusion mutant non-small cell lung cancer can be carried out. A kind of stable, universal and high efficient in vivo and in vitro gene transfection carrier used to gene therapy is made by optimizing the synthesis of gold nanoshell, the number of loaded interference genes, the needed intensity and time of illumination. As a result, this project would provide new ideas and methods of the therapy of EML4-ALK fusion mutant non-small cell lung cancer.

10066-1, Session 1

The effect of hypofractionated radiation and magnetic nanoparticle hyperthermia on tumor immunogenicity and overall treatment response *(Keynote Presentation)*

Andrew J. Giustini M.D., Stanford School of Medicine (United States); Robert J. Wagner, Rendall R. Strawbridge, Ailin Song, Bjorn Osterberg, Steven N. Fiering, P. Jack Hoopes D.V.M., Geisel School of Medicine (United States)

It is now known that many tumors develop molecular signals (immune checkpoint modulators) that inhibits an effective tumor immune response. New information also suggest that even well-known cancer treatment modalities such as radiation and hyperthermia generate potentially beneficial immune responses that have been blocked or mitigated by such immune checkpoint, or similar, molecules. The cancer therapy challenge is to; a) identify these treatment based immune signals (proteins, antigens etc), b) the treatment doses or regimens that produce them and c) the mechanism that block or has the potential to promote them. The goal of this preliminary study, using B16 murine melanoma tumor cells, the B6 mouse – B16 tumor model, clinically radiation doses/fractionation schemes (including those used clinically in hypofractionated radiation therapy), magnetic nanoparticle hyperthermia (mNPH) and sophisticated protein, immune and tumor growth analysis techniques and modulators, is to determine the effect of specific radiation and hyperthermia techniques on tumor immunity and treatment efficacy.Preliminary analysis suggest that radiation dose size (I.e.10 Gy vs 2 Gy) significantly alters the mechanism of cell death (apoptosis vs mitosis vs necrosis) and the resulting immunogenicity. Our hypothesis and data suggest this difference is protein/antigen and immune recognition based. Similarly our evidence suggest that mNP hyperthermia is immunologically different and potentially superior to other types of tumor heat therapy. Investigative tools include: cell death and immune protein/antigen identification, quantitative immunohistochemistry / pathology, in vitro colony forming assay and quantitative tumor growth kinetic analysis.

10066-2, Session 1

A novel thermal accelerant for augmentation of microwave energy during image-guided tumor ablation *(Keynote Presentation)*

William Park, Brown Univ. (United States); Aaron W. P. Maxwell, Victoria E. Frank, Michael P. Primmer, Jarod Paul, Rhode Island Hospital (United States); Cynthia Susai, Brown University (United States) and Rhode Island Hospital (United States); Tiffany M. Borjeson, Brown Univ. (United States); Greyson L. Baird, Kara A. Lombardo, Damian E. Dupuy, Rhode Island Hospital (United States)

The greatest challenge in image-guided thermal ablation (IGTA) of liver tumors is a relatively high recurrence rate (30%) due to incomplete ablation. To meet this challenge, we have developed a novel Thermal Accelerator (TA) to demonstrate its capability to, 1) augment microwave (MW) energy from a distance unreachable by antenna alone; 2) turn into a gel at body temperature; 3) act as a CT or US contrast. We examined the TA efficiency using in vitro, ex vivo and in vivo models: microwave power, TA dose, and TA-to-tip distance were varied, and temperature readings compared with and without TA. Gross pathologic analysis was performed on in vivo specimens using triphenyl tetrazolium chloride (TTC) staining to calculate ablation volumes. Using the in vitro model, both the rate and magnitude of increase in ablation zone temperature were significantly greater with TA under all tested conditions ($p<0.0001$). In vivo, liver, muscle, and kidney ablation zone volumes as determined by TTC staining were significantly increased with TA use ($p<0.01$ for all). On ultrasound imaging, the TA was echogenic as gel. On CT, TA density was proportional to dose, with average values ranging from 329 HU to 3071 HU at 10 mg/mL and 1,000mg/mL, respectively. TA can be accurately deposited to a target area using CT or US as image-guidance and augment MW energy effectively so that ablation time is significantly reduced, which will contribute to complete ablation. We are currently evaluating the ability of TA to block the “heat sink” effect, another major cause of incomplete ablation.

10066-3, Session 1

A theranostic platform for localized magnetic fluid hyperthermia and magnetic particle imaging *(Keynote Presentation)*

Daniel W. Hensley, Zhi Wei Tay, Univ. of California, Berkeley (United States); Rohan Dhavalikar, Univ. of Florida (United States); Patrick Goodwill, Magnetic Insight, Inc. (United States); Bo Zheng, Univ. of California, Berkeley (United States); Carlos Rinaldi, Univ. of Florida (United States); Steven Conolly, Univ. of California, Berkeley (United States)

Magnetic fluid hyperthermia (MFH) is a promising therapeutic approach in applications such as cancer and targeted drug delivery. A fundamental challenge in MFH is localization of the therapy deep in the body. Magnetic gradient fields provide spatial localization of the tracer signal in magnetic particle imaging (MPI) and can be leveraged to localize energy deposition in MFH. Here we demonstrate localization of MFH with a combined MPI-MFH system and explore the possibility of a new theranostic platform. A custom MPI-MFH system was built including a 2.4 T/m field-free line (FFL) quadrupole permanent magnet array and a resonant 350 kHz transmit chain connected to a solenoidal coil for homogeneous AC excitation (max amplitude approximately 20 mT). This system was used to demonstrate selective heating of individual elements of phantoms, each being 100 uL of 25 mg/mL magnetic particle samples placed approximately 7 mm apart. Heating in the range of 0.3—0.5 degrees C per second was obtained in the targeted phantom element for 30—60 seconds as measured by optical thermal probes. High quality images of the same phantom from a lower frequency MPI scanner confirmed compatibility of the magnetic particle for both MPI and MFH. Heating data, MPI relaxometer data at higher frequency (e.g., 300 kHz), and simulations all suggest that real-time SAR feedback while heating may be possible via the MPI signal. We believe this work represents the start of a powerful theranostics system capable of seamlessly transitioning between heating and imaging modes and real-time SAR feedback/imaging while heating.

10066-4, Session 1

Changes in cell mechanoelastic properties in response to nanosecond pulsed electric fields *(Keynote Presentation)*

Zachary Coker, Maria A. Troyanova-Wood, Andrew J. Traverso, Zhaokai Meng, Charles W. Ballmann, Georgi I. Petrov, Texas A&M Univ. (United States); Bennett L. Ibey, Air Force Research Lab. (United States); Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Nanosecond electric pulses (nsEPs) are known to cause a variety of effects

on mammalian cells, ranging from destabilization of cell membranes to changes in cytoskeleton and elastic moduli. Measurement of a cells mechanoelastic properties have previously been limited to only invasive and destructive techniques such as atomic force microscopy or application of optical tweezers. However, due to recent advances, Brillouin spectroscopy has now become viable as a non-contact, non-invasive method for measuring these properties in cells and other materials. Here, we present the analysis of Brillouin spectra acquired using a unique confocal microscopy system applied to CHO-K1 cells exposed to nsEPs of 60-600ns pulse durations and intensities up to 17kV/cm. Results from the Brillouin spectra analysis are consistent with previously reported atomic force microscopy measurements and demonstrate Brillouin spectroscopy as a viable method for measuring changes in elastic properties of cells and living organisms.

10066-5, Session 2

Overview of current plasma medicine applications *(Invited Paper)*

Thomas P. Ryan, Kenneth R. Stalder, Smith & Nephew, Inc. (United States)

Plasma Medicine is a rapidly growing field of treatment, with the number and type of medical applications growing annually. It combines plasma physics, life science and clinical medicine and has been shown effective against a wide range of pathogenic microorganisms. Better understanding of the basic mechanisms has evolved over time. Non-equilibrium plasmas operating at atmospheric pressure are the starting point for a variety of treatments, including antibacterial, antiviral, anti-biofilm, antifungal, and anti-spore, while sparing normal mammalian cells. The method of treatment involves exposure to highly reactive species (ions and electrons), reactive molecules, high electric field, and UV radiation, operating in a non-thermal fashion. Historically, typical applications include wound treatment and decontamination of surfaces. The range of treatments is expanding though, and now includes in-situ cancer treatment with miniaturized devices, as well as activation of nanoparticles loaded with drugs. Micron sized devices are treating cancer cells, in-situ. Work promoting muscle and blood vessel regeneration is being investigated. This review paper will cover the latest treatments using gas-based plasmas in medicine. Current clinical studies include applications in dentistry, cancer treatment, wound treatment for bacteria and fungus, prion treatment, and plasma devices for surface sterilization. Disinfection of water has been demonstrated with 8 log reduction in colony forming units. New commercial systems will also be reviewed. With the rapid increase in new investigators, development of new devices and systems for treatment, and wider clinical applications, Plasma Medicine is becoming a powerful tool in in the field of medicine.

10066-6, Session 2

Corrosion and wear in electrosurgical devices *(Invited Paper)*

Kenneth R. Stalder, Thomas P. Ryan, Jonathan Gaspredes, Jean Woloszko M.D., Smith & Nephew, Inc. (United States)

We previously reported on studies of the effects of electrical discharges on the corrosion and wear of simple, single-wire test devices immersed in isotonic saline [1]. This work showed that there are a wide variety of mechanisms that can explain various aspects of electrode mass loss, even with very simple electrode geometries and operating conditions. We found that the electrode composition played an important role. We have subsequently expanded our studies to include more realistic device geometries and operating conditions, such as suction. In the present report, we report on studies of wear characteristics of electrodes made from a variety of metals and alloys, including Waspaloy, Hastelloy, Inconel, Havar, Monel, and other pure metals such as Niobium . Depending on the operating conditions we observe multiple discrete physical and chemical effects on different locations on the surface of an individual millimeter-scale device electrode. We will present electrical data, and photographic and scanning electron micrograph (SEMs) images for a variety of test conditions and electrode materials.

1. K.R. Stalder, T.P. Ryan, J. Gaspredes, and J. Woloszko, Proc. SPIE, paper 9326-06 (2015)

10066-7, Session 2

Photocatalytic antibacterial effect of ZnO nanoparticles into coaxial electrospun PCL fibers to prevent infections from skin injuries

Gina Prado-Prone, Univ. Nacional Autónoma de México (Mexico) and Instituto Nacional de Rehabilitación (Mexico); Phaedra S. Silva-Bermúdez, Instituto Nacional de Rehabilitación (Mexico); Jorge A. García-Macedo, Argelia Almaguer-Flores, Univ. Nacional Autónoma de México (Mexico); J. Clemente Ibarra, Maria Cristina Velasquillo-Martínez, Instituto Nacional de Rehabilitación (Mexico)

Antibacterial studies of inorganic nps have become important due to the increased bacterial resistance against antibiotics. We used ZnOnps (~25nm), which possess an excellent photocatalytic property with a wide band gap (Eg ~3.2 eV at RT), are listed as a “generally recognized as safe” by Food and Drug Administration (FDA) and have shown antibacterial activity (AA) against a broad spectrum of bacterial strains. The AA of ZnOnps is attributed by its ability to produce Reactive Oxygen Species (ROS) by photocatalysis. When the ZnOnps are illuminated with an energy < Eg, electron-hole pairs are generated on nps surface reacting with H2O molecules to generate hydroxyl-radical (OH•), superoxide-radical (O2•-) and hydrogen-peroxide (H2O2). The ROS can penetrate the cell membrane resulting in cell death. However, the application of inorganic nps in medical treatment is limited due to the possible long-term side effects by nps release. To prevent its release, ZnOnps were dispersed into electrospun Polycaprolactone (PCL) fibers. In order to optimize the use of ZnOnps concentration, we developed core-shell coaxial electrospun fibers where the core corresponded to PCL and the shell to a mixture of ZnOnps/PCL. Thus, ZnOnps were only dispersed on the surface of the fibers increasing its superficial contact area. We evaluated the AA against E. coli of different electrospun ZnOnps/PCL fibers under two different pre-illumination conditions: UVA and sunlight. Preliminary results suggest that the AA is greater that antibiotic chlorhexidine gluconate and increases slightly when electrospun ZnOnps/PCL were pre-iluminated with UVA, indicating the photocatalytic antibacterial effect of ZnOnps.

10066-8, Session 3

Low intensity pulsed ultrasound (LIPUS) for the treatment of spinal disc degeneration: ultrasound exposimetry system and in vivo implementation in a rat tail model *(Invited Paper)*

Chris J. Diederich, Peter D. Jones, Devante A. Horne, Matthew S. Adams, Vasant A. Salgaonkar, Peter A. Zahos M.D., Dezba Coughlin, Stefan Dudli, Xinyan Tang, Jeff Lotz, Univ. of California, San Francisco (United States)

LIPUS or non-thermal pulsed ultrasound offers a potential treatment option for treating back pain related to intervertebral discs (IVD). Ultrasound mechanical energy targeted to a damaged IVD may produce a favorable biological response to modify, stall and even reverse IVD degeneration in a non-invasive manner. Building upon recent positive in vitro studies, the objective of our work is to develop a LIPUS exposimetry system specific to targeting degenerated disc in a rat tail model, and to apply and quantify the

effects of LIPUS on disc healing response in vivo. Ultrasound exposimetry systems were devised and fabricated specific to delivering LIPUS to damaged caudal discs in the rat tail. The two configurations consisted of 2.5 cm diameter spherically focused PZT4 transducers (1.0 MHz, f=1; 1.6 MHz, f=3.8), integrated within a water-filled 3D printed plastic housing with a mylar window, designed as an acoustic standoff to place the focus within the targeted rat tail disc. The applicators/apparatus were evaluated with comparative beam plot measurements with/without insertion of sectioned rat tails ex vivo, and demonstrated that acoustic energy is effectively delivered to the caudal disc. In vivo studies using the disc damage model were performed following standard procedures approved by UCSF IACUC, with stab incisions applied within 16 rats to generate damage/inflammation in tail discs. Five daily LIPUS exposures (ISPTA120 mW cm-2) are applied to stab discs and compared to controls. Histology and microarray gene analysis are performed at 5 and 28 days after injury, also assessing changes to cartilage, fibrous tissue, and bone. Subsequent histological and microarray analysis are currently ongoing and will be presented. This study demonstrated the technical feasibility of delivering ultrasound isolated to a targeted rat tail IVD for studies of LIPUS exposure. In summary, this LIPUS exposimetry apparatus and disc damage model can be implemented for further study in vivo to demonstrate potential of ultrasound to increase cellularity, reduce inflammation, and improve remodeling to acute or degenerative disc-related back injury. (Acknowledgment: this research was supported in part by the Focused Ultrasound Foundation.)

10066-9, Session 3

Preliminary Assessment of a Hysteroscopic Fallopian Tube Heat and Biomaterial Technology for Permanent Female Sterilization

Prajan Divakar, B. Stuart Trembly, Thayer School of Engineering at Dartmouth (United States); Karen L. Moodie, P. Jack Hoopes D.V.M., Geisel School of Medicine (United States); Ulrike Wegst, Thayer School of Engineering at Dartmouth (United States)

Purpose: The goal of this study is to assess whether a biomaterial implant can be non-invasively deposited into the fallopian tube lumen following a local mild heat treatment to generate a safe, durable, and inexpensive permanent fallopian tube occlusion/sterilization event.

Methods: Using the pre-estrous feline uterine horn (animal model for the human fallopian tube), a local intra-luminal thermal dose equivalent to 45°C for 10 min will be applied to a one centimeter region of the uterine horn mucosa prior to the deposition of a novel growth-promoting biomaterial implant. A number of techniques, including exothermic heating, microwave heating,and magnetic nanoparticle heating are assessed. The level and success of occlusion is demonstrated at 30 and 60 days post-treatment via hysterosalpingography. The uteri are removed at 60 days for histopathological assessment and confirmation of luminal occlusion.

Results: In this study, we demonstrate that the simultaneous delivery of a rapid and safe intra-fallopian tube heat dose and an occlusion-promoting biomaterial have promise as an inexpensive and effective OBGYN office-based female sterilization procedure.

10066-10, Session 3

Blue LED induced thermal effects in wound healing: experimental evidence in an in vivo model of superficial abrasions

Francesca Rossi, Istituto di Fisica Applicata “Nello Carrara” (Italy); Riccardo Cicchi, Istituto Nazionale di Ottica (Italy) and Lab. Europeo di Spettroscopie Non-Lineari (Italy); Giada Magni, Francesca Tatini, Istituto di Fisica Applicata “Nello Carrara” (Italy); Stefano Bacci, Univ. degli Studi di Firenze (Italy); Gaia Paroli, Department of Clinical and Experimental Medicine, Research Unit of Histology and Embriology (Italy); Domenico Alfieri, Cristina Tripodi, Light4Tech Firenze S.r.l. (Italy); Gaetano De Siena, Istituto di Fisica Applicata “Nello Carrara” (Italy); Francesco S. Pavone, Lab. Europeo di Spettroscopie Non-Lineari (Italy) and Istituto Nazionale di Ottica (Italy) and Univ. degli Studi di Firenze (Italy); Roberto Pini, Istituto di Fisica Applicata “Nello Carrara” (Italy)

A faster healing process was observed in superficial skin wounds after irradiation with a blue LED (EMOLED) photocoagulator. This is a compact handheld device, used to induce a thermal effect and thus coagulation in superficial abrasions. We present the results of an in vivo study in an albino mouse model, to analyze the induced wound healing. Two superficial abrasions were produced on the back of the mice: one area was treated with EMOLED (1.4 W/cm2, 30 s treatment time), while the other one was left to naturally recover. During the treatment, a temperature around 40-45°C was induced on the abrasion surface. Mice back healthy skin was used as a control. The animals underwent a follow up study and were sacrificed at 0, 1, 3, 6, 9, 12, 18, 21, 24 hours and 3, 6, 7, 15 days p.o.. Samples from the two abraded areas were harvested and examined by histopathological and immunofluorescence analysis, SHG imaging and confocal microscopy. The aim of the study was to investigate the inflammatory infiltrate, mastocyte population, macrophage subpopulation, fibroblasts and myofibroblasts. Our results show that soon after the treatment, both the inflammatory infiltrate and the M1 macrophage subpopulation appear earlier in the treated compared to untreated samples. There was no alteration in collagen morphology to wound recovered. This study confirms the preliminary results that we obtained in a rat model study: the selective photothermal effect we used for inducing immediate coagulation in superficial wounds seems to be associated to a faster and improved healing process.

10066-11, Session 3

A visible Chinese human-combined Monte Carlo simulation study on low-level light therapy of stroke

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Stroke is a devastating disease, which is the third leading cause of death and disability worldwide. Although the incidence of stroke increases progressively with age, morbidity among young and middle-aged adults is increasing annually. Medications nevertheless remain the bulwarks of stroke. The treatment is ineffective, speculative and has a long treatment cycle. Acupuncture and moxibustion exists as a potential therapeutic tool for stroke that still had a few problems. Recently, Low-level light therapy (LLLT) has demonstrated potent in vivo efficacy for treatment of ischemic conditions of acute myocardial infraction and stroke in multiple validated animal models. Optimum LLLT treatment has a dominant influence on therapy of stroke. While more than a thousand clinical trials have been halted, only a few trials on animals have been reported. We addressed this issue by simulating near-infrared light propagation within accurate visible Chinese human head by Monte Carlo modeling. The visible human head embody region of atherosclerotic plaques in the head. Through comparing the light propagation of different light illumination, we can get a precise, optimized and straightforward treatment. Here, we developed a LLLT helmet for treating stroke depend on near-infrared light. There are more than 30 LED arrays in in multi-layered 3D printed helmet. Each LED array has independent water-cooling module and can be adjust to touch the head

of different subjects based on Electro pneumatic module. Moreover, the software provides the setup of illumination parameters and 3D display of light fluence rate distribution in human brain.

10066-12, Session 4

Simple coil-powering techniques for generating 10KA/m alternating magnetic field at multiple frequencies using 0.5KW RF power for magnetic nanoparticle hyperthermia

Daqing Piao, Tengfei Sun, Ashish Ranjan, Oklahoma State Univ. (United States)

Alternating magnetic field (AMF) configurable at a range of frequencies is a critical need for optimization of magnetic nanoparticle based hyperthermia, for their application in targeted drug delivery. Currently, most commercial AMF devices including induction heaters operate at one factory-fixed frequency, thereby limiting customized frequency configuration required for triggered drug release and ablations. Nearly all AMF devices run as an inductor-capacitor resonance network that could allow AMF frequencies to be changed by changing the capacitor bank or the coil looped with it. When developing AMF in-house, the most expensive component is usually the RF power amplifier, and arguably the most critical step of building a strong AMF field is impedance-matched coupling of RF power to the coolant-cooled AMF coil. AMF devices running at 10KA/m strength are quite common, but generating AMF at that level of field strength using RF power less than 1KW has remained challenging. We practiced a few techniques for building 10KA/m AMFs at different frequencies, by utilizing a 0.5KW 80-800KHz RF power amplifier. Among the techniques indispensable to the functioning of these AMFs, a simple cost-effective technique was an adaptable rotational tapping method for continuously adjusting the position of an RF-input-tap on a single-layer or the outer-layer of a multi-layer AMF coil for maximum power coupling into the AMF coil. These in-house techniques when combined facilitated 10KA/m AMF at frequencies of 208KHz and higher as allowed by the inventory of capacitors using 0.5KW RF power, for testing drug-release by low-level temperature-sensitive liposomes loaded with 15nm magnetic nanoparticles.

10066-13, Session 4

Magneto-thermo-acoustic differential-frequency imaging of magnetic nanoparticle with magnetic spatial localization: a theoretical prediction

Daqing Piao, Oklahoma State Univ. (United States)

The magneto-thermo-acoustic effect that we predicted in 2013 refers to the generation of acoustic-pressure wave from magnetic nanoparticle (MNP) when thermally mediated under an alternating magnetic field (AMF) at a pulsed or frequency-chirped application. Several independent experimental studies have since validated magneto-thermo-acoustic effect, and a latest report has discovered acoustic-wave generation from MNP at the second-harmonic frequency of the AMF when operating continuously. We propose that applying two AMFs with differing frequencies to MNP will produce acoustic-pressure wave at the summation and difference of the two frequencies, in addition to the two second-harmonic frequencies. Analysis of the specific absorption dynamics of the MNP when exposed to two AMFs of differing frequencies has shown some interesting patterns of acoustic-intensity at the multiple frequency components. The ratio of the acoustic-intensity at the summation-frequency over that of the difference-frequency is determined by the frequency-ratio of the two AMFs, but remains independent of the AMF strengths. The ratio of the acoustic-intensity at the summation- or difference-frequency over that at each of the two second-harmonic frequencies is determined by both the frequency-ratio and the field-strength-ratio of the two AMFs. The results indicate a potential strategy for localization of the source of a continuous-wave magneto-thermal-acoustic signal by examining the frequency spectrum of full-field spatially-unresolved acoustic detection, with the field-strength ratio changed continuously at a fixed frequency-ratio. The practicalities and challenges of this magnetic spatial localization approach for magneto-thermo-acoustic imaging using a simple envisioned set of two AMFs arranged in parallel to each other are discussed.

10066-14, Session 4

Effect of intra-tumoral magnetic nanoparticle hyperthermia and viral nanoparticle immunogenicity on primary and metastatic cancer

Courtney M. Mazur, Univ. of California, Berkeley (United States); Steven N. Fiering, Geisel School of Medicine (United States); Nicole F. Steinmetz, Case Western Reserve Univ. (United States); Robert J. Wagner, Rendall R. Strawbridge, Ailin Song, Bjorn J. Osterberg, P. Jack Hoopes D.V.M., Geisel School of Medicine (United States)

In spite of the long association of medical hyperthermia and immune stimulation, the relative lack of a quantifiable and reproducible effect has limited its advancement in cancer and non-cancer settings. Recent cancer-based immune findings (immune checkpoint modulators etc) including an improved mechanistic understanding and the discovery of new immune-biological tools now make it possible to modify and exploit the immune system to improve conventional cancer treatments such as radiation and hyperthermia. Based on the prior experience of our research group; cancer-based heat therapy, magnetic nanoparticle hyperthermia (mNPH), cancer immunology/pathology and the engineering of viral nanoparticles for in vivo immune stimulation, this research demonstrates how the intra-tumoral delivery of mNPH and a modified plant virus nanoparticle (Cowpea Mosaic Virus / CPMV) can improve local and systemic tumor treatment efficacy. Primarily using the B6 mouse / B-16 murine melanoma cell model, our data suggests the appropriate combination of intra-tumoral mNP heat (e.g. 43°C /30-60 minutes) and CPMV (100 ug) not only result in significant primary tumor regression but the creation a systemic immune reaction that retards secondary tumor growth and improves tumor rechallenge kinetics. Investigative tools and assays include: immune protein/antigen identification, quantitative immune cell immunohistochemistry / pathology, ultrastructure assessment of CPMV-tumor-immune cell association, in vitro colony forming assay and quantitative tumor growth kinetic and toxicity analysis.

10066-15, Session 4

Direct and immune-based treatment efficacy of hypofractionated radiation, magnetic nanoparticle hyperthermia and a plant virus nanoparticle in the canine oral melanoma

P. Jack Hoopes D.V.M., Karen L. Moodie D.V.M., Geisel School of Medicine (United States); Alicia A. Petryk, Univ. of Bridgeport (United States); James D. Petryk, Robert J. Wagner, Rendall R. Strawbridge, Geisel School of Medicine (United States); Nichole F. Steinmetz, Case Western Reserve Univ. (United States); Steven N. Fiering, Geisel School of Medicine (United States)

We and others have recently shown that cancer treatments such as radiation

and hyperthermia, which were previously believed to have modest, largely inconsequential immune effects, have the potential to stimulate the immune system in a manner that significantly enhances both local and systemic treatment outcome. Using spontaneous canine oral melanoma cancers, we have delivered hypofractionated radiation (6 x 6 Gy), magnetic nanoparticle hyperthermia/mNPH (2 X 43°C / 45 minutes) and an immunogenic plant virus (Cowpea Mosaic Virus / CPMV (2/4 x 200 ug), alone and in combination, to determine direct and immune-based treatment efficacy. The typical treatment regimen lasts 3 - 4 weeks. Tumor tissue remaining 4 weeks after the initiation of treatment is surgically excised. Eight canine oral melanomas have been treated. The mean post treatment survival period of these dogs is significant longer (1.6X) than published survival periods for radiation and/or surgery oral melanoma treatments, however 6 / 8 patients have died of tumor progression. Immune protein/antigen identification, quantitative immune cell immunohistochemistry / pathology, quantitative primary /metastatic tumor growth analysis (CT/MRI imaging) and survival are the primary study endpoints. Preliminary data suggests that in addition to direct therapeutic efficacy, both hypofractionated radiation and mNP hyperthermia have an effective anti-cancer immune footprint and that the addition of CPMV enhances that effect. To date two dogs that have receive full course therapy (all 3 modalities). Both are in remission at 4 and 7 month, respectively. One of the dogs demonstrates marked persistent lymphoid hyperplasia in the regional (tumor) lymph nodes.

10066-16, Session 4

A ferritin-containing nanoconjugate as MRI image-guidance to target Necl-5, a tumor-surface antigen: a potential thermal accelerant for microwave ablation

William K. C. Park, David R. Mills, Brown Univ. (United States); Sierin Lim, Barindra Sana, Nanyang Technological Univ. (Singapore); Victoria E. Frank, Brendan M. Kenyon, Michael P. Primmer, Jarod B Paul, Greyson L. Baird, Rhode Island Hospital (United States); Edward Walsh, Brown University (United States); Damian E. Dupuy, Rhode Island Hospital (United States)

Purpose: A tumor-specific antigen of epithelial cancers is targeted for MRI visualization, which will be useful for improving image-guided tumor ablation as a treatment modality. In this study, we have exploited an interaction between a ferritin-containing nanoconjugate and Necl-5 (nectin-like molecule-5), a tumor-surface antigen for targeting carcinomas.

Procedure: Using an in vivo tumor model (i.e., tumor size 0.5-1 cm, immunodeficient beige/nude/xid mouse, xenograft injection with transformed rat prostate cells), efficacy of the conjugate targeting the tumor was examined. We used two injection strategies, a direct tumor injection and a tail vein injection (0.8 mg, 300 ?L per subject).

Results: The tail vein injected conjugate significantly increased R2 (1/T2) response (22.9 ± 5.2 s-1) as compared to control (13.5 ± 1.7 s-1) at 4 h. The weaker R2 increase was noted (15.2 ± 2.0 s-1) at 24 h. No notable changes in R2 were observed in surrounding tissues regardless of the stages of the measurement. Direct injection of the nanoconjugate into the center of the tumor showed a stronger and more rapid increase in R2 than the tail vein injection.

Conclusion: The nanoconjugate interacts strongly and selectively in situ with Necl-5 overexpressing tumor cells in MRI. Varying degrees of R2 increase within the tumor mass is likely to represent different distribution patterns of the conjugate, reflective of tumor heterogeneity.

10066-17, Session 5

Exploration of endoluminal ultrasound device configurations utilizing deployable arrays, reflectors and lenses to augment and dynamically adjust treatment volume, gain, and depth

Matthew S. Adams, Univ. of California, San Francisco (United States) and Univ. of California, Berkeley (United States); Vasant A. Salgaonkar, Univ. of California, San Francisco (United States); Graham Sommer, Stanford Health Care (United States); Chris J. Diederich, Univ. of California, San Francisco (United States)

Endoluminal catheter-based high intensity ultrasound offers spatially-controlled thermal ablation and hyperthermia of tissue targets adjacent to body lumens, but suffers from limited potential treatment volumes and depths due to the minimal transducer aperture sizes afforded by anatomical constraints. This study investigates various strategies of integrating endoluminal ultrasound with deployable transducer arrays, reflectors, and/or fluid lenses that can be expanded at target locations in order to increase the effective therapeutic aperture, permitting larger volumetric treatment capability or greater focal gain at target depths.

Select configurations were conceptualized and investigated. For deep and variable penetration depth with high focal gain, tubular or planar transducer sources were combined with expandable balloon acoustic reflectors and adjustable fluid lenses. For greater volumetric capabilities, deployable arrays of transducers with/without expandable reflectors were considered. Parametric studies across device design parameters were performed using acoustic simulations via the rectangular radiator method and incorporation of reflection/refraction of wave-fronts at material interfaces. Thermal modeling was used to generate resulting temperature distributions in tissue models. Proof-of-concept (POC) devices were fabricated and evaluated using hydrophone measurements to validate acoustic simulations.

Simulations indicate that configurations employing the acoustic reflector and lens achieve greater focal gain at depths beyond 2 cm as compared to a static 12 mm OD spherically-focused transducer, and that focal gain increases as a function of lens focal length to a maximum between ~20-50 mm, depending on the overall deployable reflector/lens size and tissue attenuation. Hydrophone measurements of POC assemblies demonstrate good agreement with simulated acoustic profiles.

10066-18, Session 5

Vapor ablation in the human prostate: Evolution of tissue changes over the first 90 days *(Invited Paper)*

Joshua L. Shrout, West Virginia Univ. Health Sciences Ctr. (United States); Michael F. Hoey, NxThera, Inc. (United States); James E. Coad M.D., West Virginia Univ. Health Sciences Ctr. (United States)

A variety of therapies are available for the treatment of benign and neoplastic diseases in the human prostate. Targeted vapor ablation of the diseased prostatic tissues is emerging as a clinical option for these patients. Utilizing the delayed human prostatectomy model, this study will present the evolution of the vapor ablation related tissue changes in the prostate over the first 90 days. The histology will include the progression of tissue healing, inflammation, and ability to target a control ablation within specific zones of the prostate.

10066-19, Session 5

Global microwave endometrial ablation for menorrhagia treatment

Hojjatollah Fallahi, Kansas State Univ. (United States); Jan ?ebek, Kansas State Univ. (United States) and Czech Technical Univ. in Prague (Czech Republic); Eric Frattura, Jessica Schenck, Hologic, Inc. (United States); Punit Prakash, Kansas State Univ. (United States)

Thermal ablation is a dominant therapeutic option for minimally invasive treatment of menorrhagia. Compared to other energy modalities, microwaves offer the advantages of deep penetration within tissue in short times, and the ability of achieving energy deposition patterns for delivery of conformal ablation by adapting antenna designs. The objective of endometrial ablation is to achieve a 3–8 mm ablation depth beyond the endometrium wall of the uterine cavity, with the clinical goal of achieving amenorrhea. Previous efforts have demonstrated clinical use of microwave for endometrial ablation. A considerable shortcoming of these systems is that they achieve ablation of the target by translating the applicator in a point to point fashion. Consequently, treatment outcome may be highly dependent on physician skill. Global endometrial ablation not only eliminates this operator dependence and simplifies the procedure but also facilitates shorter and more reliable treatments. The objective of our study was to investigate antenna structures and microwave energy delivery parameters to achieve global endometrial ablation. Another objective was to investigate methods for automatic and reliable determination of treatment endpoint. A 3D-coupled FEM electromagnetic heat transfer model with temperature and frequency dependent material properties was implemented to characterize microwave global endometrial ablation. The unique triangular geometry of the uterus where lateral narrow walls extend from the cervix to the fundus forming a wide base and access afforded through an endocervical approach limit the overall diameter of the final device. We investigated microwave antenna designs in a retracted state and deployed inside the uterus. The impact of microwave frequency, power level, and ablation duration on treatment outcome were investigated. Prototype applicators were fabricated and experimentally evaluated in ex vivo tissue to assess the simulation results and demonstrate proof-of-concept.

10066-20, Session 5

Considerations for ex vivo thermal tissue modeling exemplified using the fresh porcine longissimus muscle model for endometrial ablation *(Invited Paper)*

Haydon E. Bennett, Joshua L. Shrout, James E. Coad, West Virginia Univ. Health Sciences Ctr. (United States)

A large number of minimally invasive medical devices are currently under development around the world. As these devices are developed, extensive research and developmental testing is preformed using both computational, artificial constructs and most importantly ex vivo and in vivo tissue testing. A number of considerations are important to ensure that the ex vivo results are optimized to emulate the devices future use in the clinical setting. These include the proper selection of animal species, tissue type, tissue viability, tissue experimental conditions, viability stain selection and interpretation. These issues will be illustrated using the fresh extirpated porcine longissimus muscle model for endometrial ablation.

10066-21, Session 5

Flexible microwave ablation applicator for the treatment of pulmonary malignancies

Austin Pfannenstiel, Kansas State Univ. (United States); Thomas Keast, Steve Kramer, Henky Wibowo, Broncus Medical, Inc. (United States); Punit Prakash, Kansas State Univ. (United States)

Microwave ablation (MWA) is an emerging minimally invasive treatment option for malignant lung tumors. Compared to other energy modalities, such as radiofrequency ablation, MWA offers the advantages of deeper penetration within high impedance tissues such as aerated lung, shorter treatment times, and is also less susceptible to the cooling heat-sink effects of air and blood flow. Previous studies have demonstrated clinical use of MWA for treating lung tumors, however, these procedures have relied upon the percutaneous application of rigid microwave antennas. The objective of our work was to develop and characterize a novel flexible microwave applicator which could be integrated with a bronchoscopic imaging and software guidance platform to expand the use of MWA as a treatment option for small (< 2 cm) pulmonary tumors. This applicator would allow physicians an even less invasive, immediate treatment option for lung tumors identified within the scope of current medical procedures, improve applicator placement accuracy and may increase efficacy while minimizing the risk of procedural complications. A 2D-axisymmetric coupled FEM electromagnetic-heat transfer model was implemented to characterize expected antenna radiation patterns, ablation size and shape, and optimize antenna design for lung tissue. Multiple coaxial cable types, applicator cooling schemes, power levels, and treatment durations were evaluated in ex-vivo tissues to verify simulation results and serve as proof-of-concept. Additional experiments were conducted in vivo to further characterize the proposed system.

10066-22, Session 5

Methods to adjust heating patterns of microwave waveguide applicators using a conformal water bolus *(Invited Paper)*

Paul R. Stauffer, Dario B. Rodrigues, Thomas Jefferson Univ. (United States); Arulselvie Selvaraja, Drexel Univ. (United States); Mark D. Hurwitz M.D., Thomas Jefferson Univ. (United States)

Background:

Hyperthermia, i.e. raising tissue temperature to 40-45C, has been demonstrated to increase the effectiveness of radiation and chemotherapy for cancer. Although conformal heat applicators are under development to provide better coupling to contoured anatomy, to date the most often used applicator to heat superficial disease is the simple microwave waveguide. With only a single power input, the operator must be proactive to adjust heat treatment to accommodate variable size, shape, and depth of tumors spreading across contoured tissue surfaces. Waveguide heating may be adjusted via changes in power, position, and waterbolus coupling layer size, thickness and temperature.

Methods:

We use multiphysics software that couples electromagnetic, thermal and fluid flow physics to simulate heating patterns in superficial tumors from commercially available microwave waveguide applicators. Temperature distributions are calculated inside layered fat-muscle-tumor tissue loads for a typical range of water bolus size, thickness and temperature. Variable thickness waterbolus is also simulated as needed to accommodate contoured anatomy.

Results:

We demonstrate a wide range of power deposition patterns possible from commercially available waveguide antennas by controllably varying size, thickness and temperature of the waterbolus layer. Results of parametric studies provide useful guidance on use of a waterbolus to adjust heating within 40-45?C under a waveguide aperture.

Conclusion:

Lateral and depth heating characteristics of 915MHz waveguide antennas can be varied over a wide range by controlled adjustment of waterbolus

thickness and temperature to accommodate patient pain while providing higher temperatures and more uniform thermal dose coverage of tissues across the aperture.

10066-23, Session 6

Association of continuous changes in optical properties to thermal damage during thermal ablation of ex vivo porcine liver

Vivek Krishna Nagarajan, Bing Yu, The Univ. of Akron (United States)

More than two-thirds of patients with primary liver cancer and 90 percent of patients with secondary liver cancer have large tumor size or tumors located in close proximity to critical structures, making them ineligible for surgical resection. Thermal ablation procedures are increasingly being used to treat such inoperable hepatic tumors. However, incomplete ablation rates for radiofrequency ablation of hepatocellular carcinoma can be as high as 52%. Therefore, continuous monitoring of a thermal ablation process is vital for achieving complete tumor ablation. Quantitative diffuse reflectance spectroscopy (DRS) is a non-destructive method that is sensitive to tissue absorption and scattering and can be used to quantify tissue morphological and physiological properties during thermocoagulation. In this paper, a portable DRS system (430-630 nm) consisting of an integrated fiber-optic probe with a self-calibration channel, two DRS fibers and an interferometric temperature sensor was developed to measure the continuous changes in wavelength-averaged tissue absorption (<μa(?)>) and reduced scattering coefficients (<μ's(?)>) and local tissue temperature (T) during heating of ex vivo porcine liver tissues. The mean <μa(?)> increased from 8.4±0.8 cm-1 at 37°C to 16.8±2.5 cm-1 at 70°C. The mean <μ's(?)> increased from 3.2±0.5 cm-1 at 37°C to 6.2±1.3 cm-1 at 70°C. Further investigations includes association of the thermal damage calculated from Arrhenius model and from histological assessment to changes in <μa(?)> and <μ's(?)>.

10066-24, Session 6

Corrections for ultrasound image guided therapies using electromagnetic tracking: steps toward stereotactic procedures and in vivo speed of sound measurements

Vishal Samboju, Matthew S. Adams, Vasant A. Salgaonkar, Chris J. Diederich, J. Adam M. Cunha, Univ. of California, San Francisco (United States)

The speed of sound (SOS) for ultrasound (US) devices used for imaging soft tissue is often calibrated to water, 1540 m/s, despite actual in-vivo soft tissue SOS varying from 1450 to 1613 m/s. Images acquired with a SOS based on 1540 m/s and used in conjunction with a stereotactic external coordinate system may result in image distortion and displacement errors of several millimeters. Brachytherapy uses small radioactive pellets, inserted interstitially with needles under ultrasound guidance, to eradicate cancerous tissue. Since the photon flux diminishes with distance from the pellet as 1/r2, imaging uncertainty of a few millimeters can result in significant erroneous dose delivery in US-guided radiotherapy.

This work presents a method of mitigating needle trajectory error due to SOS variances. It establishes the proof of principle of in-vivo SOS measurement using electromagnetic tracking (EM) measurements and algorithms to generate corrected b-mode US images. We will demonstrate the effects of changes in dosimetry due to patient-specific SOS variances and the ability to mitigate this dose delivery uncertainty.

EM probes embedded in the trans rectal ultrasound (TRUS) system provide information regarding position and orientation of the sensors. Correctional algorithms have been developed using data from these two modalities to provide corrected b-mode images. EM tracking resolution was verified to <1 mm precision while US localization resulted in >3 mm displacements from physical measurement using custom-built phantoms with SOS ranging between 1440 and 1670 m/s in clinical brachytherapy environments. Results show 1% accuracy in SOS measurement.

10066-25, Session 6

Longitudinal assessment of single-dose radiation-induced tumor vascular changes with optical coherence tomography

Valentin Demidov, Univ. of Toronto (Canada); Azusa Maeda, Princess Margaret Cancer Ctr. (Canada); Egor Demidov, Saratov State Univ. (Russian Federation); Ralph S. DaCosta, Univ. of Toronto (Canada) and Princess Margaret Cancer Ctr, Univ. Health Network (Canada); I. Alex Vitkin, Univ. of Toronto (Canada) and Princess Margaret Cancer Ctr. (Canada)

We report on optical coherence tomography (OCT) quantitative assessment of early (up to 4 weeks) microvascular response of engrafted tumors in mouse dorsal skin window chamber model subjected to single-fraction radiation treatment (8, 15, 20, and 30Gy doses; NRG mice; Bx-PC3 human pancreatic xenografts).

Optimized speckle variance OCT imaging of microvasculature was performed before and after each treatment (several time points). Imaging was followed by OCT data pre- and post-processing to characterize the structure and function of tumor-associated and normal vasculatures. Several biological metrics were used to quantify early microvascular changes in response to radiotherapy. Vascular damage, angiogenesis, neovascularization and changes in vessel diameters were estimated with vascular volumetric and length densities. Average vessel length metric was used to quantify vessel/capillary pruning. Vessel tortuosity reflected efficiency of blood transport and vascular remodeling. Finally, microvasculature fractal dimension characterized changes in vascular space-filling properties and vascular network complexity through box-counting method.

This study demonstrates OCT's potential to monitor and characterize early microvascular changes in irradiated tissues. Obtained results are compared with literature on other imaging modalities, and their interpretation in terms of underlying microvascular radiobiological changes is provided.

10066-26, Session 7

Complications of vessel architecture and the characteristics of cylindrical electrodes *(Invited Paper)*

John A. Pearce, Sharon Thomsen, The Univ. of Texas at Austin (United States)

Large vessels can be reliably sealed with radio frequency current. High apposition pressures are necessary to ensure a high probability of a successful seal. However, the complex architecture of the vessels, particularly arteries, means that results can vary substantially even with similar thermal histories. The relative volume fractions and spatial distributions of collagen, elastin, and smooth muscle dominate the vessel function in vivo and can even vary from proximal to distal locations in the same vessel. We begin by reviewing the architectural features characteristic of porcine and canine large vessels, and conclude with an experimental and numerical modeling demonstration of the characteristics of cylindrical RF electrodes.

10066-27, Session 7

Histological evaluation and optimization of surgical vessel sealing systems

Robert Lathrop, Thomas P. Ryan, Jonathan Gaspredes, Jean Woloszko M.D., Smith & Nephew, Inc. (United States); James E. Coad M.D., West Virginia Univ. Health Sciences Ctr. (United States)

Surgical vessel sealing systems are widely used to achieve hemostasis and dissection in open surgery and minimally invasive, laparoscopic surgery. This enabling technology was developed about 17 years ago and continues to evolve with new devices and systems achieving improved outcomes. Histopathological assessment of thermally sealed tissues is a valuable tool for refining and comparing performance among surgical vessel sealing systems. Early work in this field typically assessed seal time, burst pressure, and failure rate (in-situ). Later work compared histological staining methods with birefringence to assess the extent of thermal damage to tissues adjacent to the device. Understanding the microscopic architecture of a sealed vessel is crucial to optimizing the performance of power delivery algorithms and device design parameters. Manufacturers rely on these techniques to develop new products. A system for histopathological evaluation of vessels and sealing performance was established, to enable the direct assessment of a treatment's tissue effects. The parameters included the commonly used seal time, burst pressure and failure rate, as well as extensions of the assessment to include its likelihood to form steam vacuoles, adjacent thermal effect near the device, and extent of thermally affected tissue extruded back into the vessel lumen. This comprehensive assessment method provides an improved means of assessing the quality of a sealed vessel and understanding the exact mechanisms which create an optimally sealed vessel.

10066-28, Session 7

A hand-held high energy delivery laser scalpel system for laser microsurgery

Kaushik Subramanian, Ilan Gabay, The Univ. of Texas at Austin (United States); Michal E. Pawlowski, Tomasz S. Tkaczyk, Rice Univ. (United States); Adela Ben-Yakar, The Univ. of Texas at Austin (United States)

We present the development of a fully hand-held, 5 mm, piezo-actuated, ultrafast laser scalpel for microsurgery with a capability to deliver energies in excess of 1 ?J per pulse that we achieved in our previous surgery probes. High energy fiber delivery is possible thanks to the use of a large, 31 ?m cored inhibited-coupling kagome fiber. Piezoelectric fiber tip actuation is used to achieve large scan area by driving the fiber tip at resonance. The design also incorporates a plug and play modular objective design, for easy replacement of objective. The custom CaF2 objective boasts diffraction limited performance over the full field of view of 100 ?m. Our previous iteration used ZnS lenses for the objective. However, we encountered undesirable non-linear effects in the lenses due to the large non-linear refractive index. Using Calcium Fluoride lenses allowed higher peak powers to be transmitted through the system without unwanted non-linear absorption and self-focusing effects. Here, we compare and contrast the performance of the two objectives. Additionally, the CaF2 probe's performance will be tested via metal and tissue ablation studies, characterizing high speed ablation parameters and uniformity of ablation over the scan area.

10066-29, Session 7

Tissue dissection using a 1470-nm diode laser and laparoscopic prototype

Chun-Hung Chang, The Univ. of North Carolina at Charlotte (United States); John Hammerland, William H. Nau Jr., Medtronic, Inc. (United States); Nathaniel M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: The 1470-nm, continuous-wave, diode laser may represent a compact inexpensive alternative to pulsed Holmium:YAG laser (wavelength=2120 nm) for tissue dissection. Both lasers wavelengths have similar, ~400 micrometer optical penetration depths. This study explores 1470-nm laser for dissection of thin, fascia layers for potential use in laparoscopic surgery.

Methods: A 40-Watt, 1470-nm diode laser delivered energy through 550-micrometer-core silica fiber, inside 5-mm-outer-diameter, laparoscopic probe (Maryland jaw configuration) for future integration into conventional electrosurgical instruments. A detachable, 2-mm-diameter, sapphire ball rolling probe tip was used for contact tissue ablation, and to focus beam to approximately 600-micrometer-diameter spot on tissue surface. Porcine mesentery fascia of 100 micrometer thickness was used, ex vivo. The probe was mounted on a linear stage, and laser power (13-20 W) and scan speed (1.0-2.75 mm/s) studied for creating 2-cm-long incisions. A thermal camera and micro-thermocouples mounted on jaws measured peak temperatures and cooling times before and after laser activation.

Results: Laser probe cleanly dissected fascia at speed of 2.5 mm/s using 16 W. Collateral thermal damage at cut edges measured 340±70 micrometer. Up to 3 incisions were made before sapphire ball needed cleaning or replacement. Peak temperatures measured approximately 130 C at ball tip and 75 C along metal jaws.

Conclusions: The 1470-nm laser rapidly and precisely dissects fascia with minimal thermal damage. Peak jaw temperatures are less than 100-200 C observed with conventional radiofrequency and ultrasound instruments. With further development, 1470-nm laser may represent alternative to Holmium as multi-purpose laser for tissue cutting and coagulation.

10066-30, Session 8

The influence of medium conductivity on cells exposed to nsPEF

Erick K. Moen, The Univ. of Southern California (United States); Bennett L. Ibey, Caleb C. Roth, Air Force Research Lab. (United States); Ronald A. Barnes Jr., Oak Ridge Institute for Science and Education (United States); Hope T. Beier, Air Force Research Lab. (United States); Andrea M. Armani, The Univ. of Southern California (United States)

Nanosecond pulsed electric fields (nsPEF) have proven useful for transporting cargo across cell membranes and selectively activating cellular pathways. The chemistry and biophysics governing this cellular response, however, are complex and not well understood. Recent studies have shown that the conductivity of the solution cells are exposed in could play a significant role in plasma membrane permeabilization and, thus, the overall cellular response. Unfortunately, the means of detecting this membrane perturbation has traditionally been limited to analyzing one possible consequence of the exposure – diffusion of molecules across the membrane. This method has led to contradictory results with respect to the relationship between permeabilization and conductivity. Diffusion experiments also suffer from "saturation conditions" making multi-pulse experiments difficult. As a result, this method has been identified as a key stumbling block to understanding the effects of nsPEF exposure. To overcome these limitations, we recently developed a nonlinear optical imaging technique based on second harmonic generation (SHG) that allows us to identify nanoporation in live cells during the pulse in a wide array of conditions. As a result, we

are able to explore and fully test whether lower conductivity extracellular solutions could induce more efficient nanoporation. This hypothesis is based on membrane charging and the relative difference between the extracellular solution and the cytoplasm. The experiments also allow us to test the noise floor of our methodology against the effects of ion leakage. The results emphasize that the electric field, not ionic phenomenon, are the driving force behind nsPEF-induced membrane nanoporation.

10066-31, Session 8

Nanosecond electric pulses modulate skeletal muscle calcium dynamics and contraction

Christopher Valdez, Michael Jirjis, Caleb C. Roth, Ronald A. Barnes Jr., Bennett L. Ibey, Air Force Research Lab. (United States)

Irreversible electroporation therapy is utilized to remove cancerous tissues thru the delivery of rapid (250Hz) and high voltage (1500V/cm) electric pulses across microsecond durations. Clinical research demonstrated that biphasic high voltage microsecond pulses opposed to monophasic waveforms relieve muscle contraction during electroporation treatment. Our group along with others discovered that nanosecond electric pulses (nsEP) can activate second messenger cascades, induce cytoskeletal rearrangement, and depending on the nsEP duration and frequency, initiate apoptotic pathways. Of high interest across in vivo and in vitro applications, is how nsEP affects muscle physiology, and if nuances exist in comparison to longer duration electroporation applications. To this end, we exposed mature skeletal muscle cells to mono and biphasic nsEP stimulation across a wide range of electric field amplitudes (1-20 kV/cm). From live confocal microscopy, we simultaneously monitored intracellular calcium dynamics along with nsEP-induced muscle movement on a single cell level. In addition, we also evaluated membrane permeability with Yo-PRO®- 1 and Propidium Iodide (PI) across various nsEP parameters. The results from our findings suggest that skeletal muscle calcium dynamics, and nsEP-induced contraction exhibit exclusive responses to both mono and biphasic nsEP exposure. Overall the results suggest in vivo nsEP application may elicit unique physiology and field applications compared to longer wavelength electroporation.

10066-32, Session 8

A new paradigm for use of ultrafast lasers in ophthalmology for enhancement of corneal mechanical properties and permanent correction of refractive errors

Mikhail A. Fomovsky, Chao Wang, Jamie R. A. Hall, David C. Paik, Stephen L. Trokel M.D., Sinisa Vukelic, Columbia Univ. (United States)

A new paradigm for strengthening of corneal tissue as well as permanent correction of refractive errors has been proposed. Ultrafast laser irradiation is confined to the levels below optical breakdown such that tissue damage is avoided while creating an ionization field responsible for subsequent physical modification of the stroma. The concept was assed using newly developed R&D platform for precise application of a near-IR femtosecond laser irradiation to the cornea in in vitro experiments. Targeted irradiation with tightly focused ultrafast laser pulses allows spatially resolved crosslinking in the interior of the porcine cornea without aid of photosensitizers. The results of electron paramagnetic resonance spectroscopy (EPR), Differential Scanning Calorimetry (DSC) and Fluorescent Spectrometry analysis demonstrated that ionization induced by femtosecond oscillator is responsible for creation of reactive oxygen species (ROS) initiating formation of covalent bonds between stromal collagen fibrils and increasing corneal rigidity. As the induced modification is primarily driven by nonlinear absorption, the treatment is essentially wavelength independent, and as such potentially less harmful than current method of choice, joint application of UVA light irradiation in conjunction with riboflavin. Potential applicability of a near-IR femtosecond laser for biomechanical stabilization of cornea and non-invasive refractive eye corrections is discussed.

10066-33, Session 8

A computerized tutor prototype for prostate cryotherapy: key building blocks and system evaluation *(Invited Paper)*

Yoed Rabin, Carnegie Mellon University (United States) and The STAR Center, Allegheny Health Network (United States); Kenji Shimada, Purva Joshi, Anjali Sehrawat, Robert Keelan, Carnegie Mellon Univ. (United States); Dona M. Wilfong, The STAR Ctr., Allegheny Health Network (United States); James T. McCormick, Allegheny General Hospital (United States)

The current presentation focuses on the evaluation of a prototype for computer-based tutoring of prostate cryosurgery. The tutoring system lists geometrical constraints of cryoprobes placement, simulates cryoprobe insertion, displays a rendered shape of the prostate, enables distance measurements, simulates the corresponding thermal history, and evaluates the mismatch between the target region shape and a pre-selected planning isotherm. The quality of trainee planning is measured in comparison with a computer-generated planning, created for each case study by previously developed planning algorithms. While the tutoring level in this study aims only at geometrical constraints on cryoprobe placement and the resulting thermal histories, it creates a unique opportunity to gain insight into the process outside of the operation room. System validation has been performed by collecting training data from surgical residents, having no prior experience or advanced knowledge of cryotherapy. Furthermore, the system has been evaluated by graduate engineering students having no formal background in medicine. In terms of match between a planning isotherm and the target region shape, results demonstrate medical residents' performance improvement from 4.4% in a pretest to 44.4% in a posttest over a course of 50 minutes of training. In terms of combined performance, including geometrical match and constraints on cryoprobe placement, this study demonstrates medical residents' performance improvement from 2.2% in the pretest to 31.1% in the posttest. Comparing those results with the performance of engineering students indicates that planning of the cryoprobe layout essentially revolves around geometric considerations.

Conference 10067: Optical Elastography and Tissue Biomechanics IV

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10067-1, Session 1

Air-coupled acoustic radiation force for non-contact OCE

Soon Joon Yoon, Shaozhen Song, Univ. of Washington (United States); Lukasz Ambrozi?ski, Univ. of Washington (United States) and AGH Univ. of Science and Technology (Poland); Ivan M. Pelivanov, Univ. of Washington (United States) and M.V. Lomonosov Moscow SU (Russian Federation); David S. Li, Liang Gao, Tueng T. Shen, Ruikang K. Wang, Matthew O'Donnell, Univ. of Washington (United States)

Over the past decades, elastography techniques have been investigated to map the elasticity of soft media. However, most elastography methods use contact or minimally-invasive excitations to generate mechanical waves. In this study, we propose a new non-contact and non-invasive method of mechanical wave excitation for elasticity imaging in general, and optical coherence elastography (OCE) in particular. To demonstrate this approach, we have designed and fabricated a home-made, focused 1 MHz air-coupled piezoelectric transducer with a matching layer to launch an ultrasound (US) wave through air onto the sample surface. To provide an acoustic line source approximating a 1-D excitation, the transducer was made from a cylindrical segment of a piezoelectric tube. A chirp signal ranging from 0.95-1.05 MHz, with an amplitude of 250 V peak to peak and 200 μs in duration, was applied to the air-coupled transducer to minimize standing wave effects between the transducer and the sample surface. The resultant US intensity profile was also characterized. A phase-sensitive optical coherence tomography (PhS-OCT) system was utilized to measure acoustic wave propagation in a pig cornea at different intraocular pressures (IOPs). Local displacements were calculated based on speckle tracking, and elasticity maps were subsequently reconstructed from displacement fields using a time-of-flight algorithm. Results from this OCE study demonstrate that an air-coupled US wave reflected from an air/tissue interface provides significant radiation force to generate displacement for elasticity imaging.

10067-2, Session 1

Longitudinally polarized shear wave optical coherence elastography

Yusi Miao, Univ. of California, Irvine (United States); Jiang Zhu, Li Qi, Beckman Laser Institute and Medical Clinic (United States); Yueqiao Qu, Youmin He, Yiwei Gao, Univ. of California, Irvine (United States); Zhongping Chen, Beckman Laser Institute and Medical Clinic (United States) and Univ. of California, Irvine (United States)

Shear wave measurement enables quantitative assessment of tissue viscoelasticity. In previous studies, a transverse shear wave was measured using optical coherence elastography (OCE), which gives poor resolution along the force direction because the shear wave propagates perpendicular to the applied force. In this study, for the first time to our knowledge, we introduce an OCE method to detect a longitudinally polarized shear wave that propagates along the force direction. The direction of vibration induced by a piezo transducer (PZT) is parallel to the direction of wave propagation, which is perpendicular to the OCT beam. A Doppler variance method is used to visualize the transverse displacement. Both homogeneous phantoms and a side-by-side two-layer phantom were measured. The elastic moduli from mechanical tests closely matched to the values measured by the OCE system. Furthermore, we developed 3D computational models using finite element analysis to confirm the shear wave propagation in the longitudinal direction. The simulation shows that a longitudinally polarized shear wave is present as a plane wave in the near field of planar source due to diffraction effects. This imaging technique provides a novel method for the assessment of elastic properties along the force direction, which can be especially useful to image a layered tissue.

10067-3, Session 1

Dual-scanning optical coherence elastography for rapid imaging of two tissue volumes

Qi Fang, Luke Frewer, Harry Perkins Institute of Medical Research (Australia); Philip Wijesinghe, The Univ. of Western Australia (Australia); Juliana Hamzah, Ruth Ganss, Wes M. Allen, Harry Perkins Institute of Medical Research (Australia); David D. Sampson, Andrea Curatolo, The Univ. of Western Australia (Australia); Brendan F. Kennedy, Harry Perkins Institute of Medical Research (Australia)

In many applications of optical coherence elastography (OCE), it is necessary to rapidly acquire images in vivo, or within intraoperative timeframes, over fields-of-view far greater than can be achieved in one OCT image acquisition. For example, tumour margin assessment in breast cancer requires acquisition over linear dimensions of 4-5 centimetres in under 20 minutes. However, the majority of existing techniques are not compatible with these requirements, which may present a hurdle to the effective translation of OCE. To increase throughput, we have designed and developed an OCE system that simultaneously captures two 3D elastograms from opposite sides of a sample. The optical system comprises two interferometers: a common-path interferometer on one side of the sample and a dual-arm interferometer on the other side. This optical system is combined with scanning mechanisms and compression loading techniques to realize dual-scanning OCE. The optical signals scattered from two volumes are simultaneously detected on a single spectrometer by depth-encoding the interference signal from each interferometer. To demonstrate dual-scanning OCE, we performed measurements on tissue-mimicking phantoms containing rigid inclusions and freshly isolated samples of murine hepatocellular carcinoma, highlighting the use of this technique to visualise 3D tumour stiffness. These findings indicate that our technique holds promise for in vivo and intraoperative applications.

10067-4, Session 2

Measurement of tissue viscoelasticity with ultrasound *(Keynote Presentation)*

James F. Greenleaf, Azra Alizad M.D., Mayo Clinic (United States)

Tissue properties such as elasticity and viscosity have been shown to be related to such tissue conditions as contraction, edema, fibrosis, and fat content among others. Magnetic Resonance Elastography has shown outstanding ability to measure the elasticity and in some cases the viscosity of tissues, especially in the liver, providing the ability to stage fibrotic liver disease similarly to biopsy. This talk will discuss the methods and applications of ultrasound shear wave elastography for measuring elasticity and viscosity in tissues. Many of these methods are becoming widely available in the extant ultrasound machines available throughout the world. Some of the methods to be discussed are in the developmental stage. The advantages of the ultrasound methods are that the machines are widely available and that many of the viscoelastic measurements can be made as a short addition to the normal ultrasound examination time. In addition, the measurements can be made by ultrasound repetitively and quickly allowing

the evaluation of dynamic physiologic function in circumstances such as muscle contraction or artery relaxation. Measurement of viscoelastic tissue mechanical properties will become a consistent part of clinical ultrasound examinations in our opinion.

10067-5, Session 3

Recent advances in optical coherence elastography of ocular and cardiac tissues *(Invited Paper)*

Kirill V. Larin, Univ. of Houston (United States)

Optical coherence elastography (OCE) is relatively new emerging method allowing to assess biomechanical properties of tissues in situ and in vivo in 3D. In this talk I will overview recent progress made in the quantitative assessment of viscoelasticity of ocular and cardiac tissues. Low-amplitude elastic deformations in mice and rabbit ocular tissues and mice hearts (both ex vivo and in vivo) were measured by the OCE system consisting of a phase-sensitive optical coherence tomography (OCT) combined with focused ultrasound (lens excitation) or air-puff (cornea and heart muscle excitation) systems used to produce a transient force on the tissue surface. The amplitude, temporal profile, and the speed of the deformations were used to reconstruct tissue biomechanical properties using novel analytical models. The results of these studies demonstrate that the OCE system can be used for noninvasive analysis and quantification of tissue biomechanical properties in 2D and 3D in normal and pathological tissues and as a function of tissue aging or therapy (e.g. CLX procedures). At the end, I'll introduce our recent advances in ultra-high speed imaging and assessment of the elastic waves using several configurations such as MHz laser swept source and optimizing scanning/imaging methods (such line-field low-coherence holography).

10067-7, Session 4

Passive optical coherence elastography using a time-reversal approach

Thu-Mai Nguyen, Institut Langevin (France); Ali Zorgani, Lab d'Applications thérapeutiques des ultrasons (France); Mathias Fink, Institut Langevin (France); Stefan Catheline, Lab d'Applications thérapeutiques des ultrasons (France); A. Claude Boccara, Institut Langevin (France)

Background and motivation -

Conventional Optical Coherence Elastography (OCE) methods consist in launching controlled shear waves in tissues, and measuring their propagation speed using an ultrafast imaging system. However, the use of external shear sources limits transfer to clinical practice, especially for ophthalmic applications. Here, we propose a totally passive OCE method for ocular tissues based on time-reversal of the natural vibrations.

Methods -

Experiments were first conducted on a tissue-mimicking phantom containing a stiff inclusion. Pulsatile motions were reproduced by stimulating the phantom surface with two piezoelectric actuators excited asynchronously at low frequencies (50-500 Hz). The resulting random displacements were tracked at 190 frames/sec using spectral-domain optical coherence tomography (SD-OCT), with a 10x5μm? resolution over a 3x2mm? field-of-view (lateral x depth). The shear wavefield was numerically refocused (i.e. time-reversed) at each pixel using noise-correlation algorithms. The focal spot size yields the shear wavelength. Results were validated by comparison with shear wave speed measurements obtained from conventional active OCE. In vivo tests were then conducted on anesthetized rats.

Results -

The stiff inclusion of the phantom was delineated on the wavelength map with a wavelength ratio between the inclusion and the background (1.6) consistent with the speed ratio (1.7). This validates the wavelength measurements. In vivo, natural shear waves were detected in the eye and wavelength maps of the anterior segment showed a clear elastic contrast between the cornea, the sclera and the iris.

Conclusion -

We validated the time-reversal approach for passive elastography using SD-OCT imaging at low frame-rate. This method could accelerate the clinical transfer of ocular elastography.

10067-8, Session 4

Optical coherence elastography reveals the spatial stiffness of porcine heart valves

Manmohan Singh, Univ. of Houston (United States); Dessy Vekilov, Rice Univ. (United States); Raksha Raghunathan, Univ. of Houston (United States); Jane Grande-Allen, Jane Grande-Allen, Rice Univ. (United States); Kirill V. Larin, Univ. of Houston (United States) and Baylor College of Medicine (United States)

Cardiac valves perform a critical task by maintaining unidirectional flow in the heart, and their biomechanical properties are critical for healthy function. Moreover, one of the major areas of bioengineering is development of heart valves with biomechanical properties that match native heart valves. Thus, assessing the biomechanical properties of heart valves can provide critical information for detecting the onset of disease and is crucial for development of effective heart valve replacements. In this work, we utilize noncontact elastic wave imaging optical coherence elastography (EWI-OCE) to quantify the spatial stiffness of pig mitral valves. By analyzing the dispersion curve of the elastic wave propagation, the viscoelasticity was quantified by a Rayleigh-Lamb wave model. The results show that the stiffness and shear viscosity of the collagen-rich annular region of the anterior leaflet was the greatest (~16 kPa and ~5 Pa•s as compared to ~6 kPa and ~1 Pa•s in the glycosaminoglycan-rich distal region). The posterior leaflet was more elastic with a Young's modulus of ~4 kPa and shear viscosity of ~1 Pa•s, and the posterior leaflet exhibited a lesser degree of viscoelastic variation. The results corroborated well with mechanical extensiometry, showing that EWI-OCE is an effective method of assessing cardiac valve mechanical properties.

10067-9, Session 4

In situ dynamic mechanical analysis of heterogeneous soft tissue using a 1mm probe

Steven V. Beekmans, Davide Iannuzzi, Vrije Univ. Amsterdam (Netherlands)

The mechanical properties of biological tissue play an important role in both the development and functioning of the tissue and can vary widely within a sample. There is a strong need for the local quantification of tissue mechanics at time scales relevant to biological processes. The available techniques for quantifying mechanical properties are either limited in spatial orientation (large tip size) or are unable to give depth resolved mechanical information (low penetration depth). Here, we present a nano-indentation device, actuated at the tip of a minimally invasive needle (diameter = 1 mm), that is engineered to quantify viscoelastic properties of soft, hydrated tissues. The probe, driven by a feedback-controlled piezo-electric actuator, employs the bending of a micro-machined cantilever fabricated on top of an optical fiber. The displacement of the cantilever, imposed by pressing a micro-bead (r = 60 μm) glued at the tip of the cantilever against the tissue, is interrogated by Fabry-Pérot interferometry and converted to load acted on the tissue in real-time. The elastic and viscous moduli of the tissue are obtained by superimposing a series of sinusoidal loads of increasing

frequency on top of the feedback-controlled static force. In this work, we show the potential of our device to detect and identify different biological tissues on the basis of their viscoelastic properties by means of localized, in situ measurements.

10067-10, Session 4

Mapping the mechanical heterogeneity of the brain, and why this matters *(Invited Paper)*

Jochen R. Guck, TU Dresden (Germany)

It is increasingly recognized that cells measure and respond to the mechanics of their environment. We are especially interested in this mechanosensing during CNS development and pathologies. Using quantitative scanning force microscopy we have shown that various neural tissues are very compliant (shear modulus < 1 kPa) and mechanically heterogeneous. We have recreated compliant polyacrylamide gel substrate with shear moduli between 0.1 and 30 kPa to match and exceed those of CNS tissue. Various primary neurons and glial cells have been cultured on these gels and their reaction studied. Both primary microglia and astrocytes responded to increasing substrate stiffness by changes in morphology and upregulation of inflammatory genes. Upon implantation of composite hydrogel stripes into rat brains, foreign body reactions were significantly enhanced around the stiff parts of the implant. It appears that the mechanical mismatch between a neural implant and native tissue might be at the root of foreign body reactions. Also oligodendrocytes are mechanosensitive as their survival, proliferation, migration, and differentiation capacity in vitro depend on substrate stiffness. This finding might be linked to the failure of remyelination in chronic demyelinating diseases such as multiple sclerosis. And finally, we have also shown retinal ganglion axon pathfinding in the early embryonic Xenopus brain development to be instructed by stiffness gradients. These results form the basis for further investigations into the mechanobiology of cell function in the CNS. Ultimately, this research could help treating previously incurable neuropathologies such as spinal cord injuries and neurodegenerative disorders.

10067-11, Session 5

OCT-based elastography to study in-vivo the interplay between intraocular and intracranial pressure effects on the posterior pole of the eye *(Invited Paper)*

Ian A. Sigal, Huong Tran, Bo Wang, Matthew A. Smith, Univ. of Pittsburgh School of Medicine (United States); Joel S. Schuman M.D., Gadi Wollstein M.D., Univ. of Pittsburgh School of Medicine (United States) and New York Univ. (United States)

Although it is well documented that abnormal levels of either intraocular or intracranial pressure can lead to potentially blinding conditions, such as glaucoma and papilledema, little is known about how the pressures actually affect the eye. Even less is known about potential interplays in their effects, namely how the level of one pressure might alter the effects of the other. Our goal was to use elastography based on optical coherence tomography to measure with high detail the in-vivo biomechanical effects on the posterior pole of the eye of acute controlled changes in intraocular and/or intracranial pressures.

Eight eyes of five macaque monkeys were imaged in-vivo with optical coherence tomography while intraocular and intracranial pressures were controlled through cannulas in the anterior chamber and lateral ventricle, respectively. The image volumes were analyzed with a combination of manual delineations and a custom tracking algorithm based on image registration. The effects of both pressures were nonlinear and non-monotonic, with strong interactions. Pressure variations from the baseline normal levels caused substantial stretch and compression of the neural tissues in the posterior pole, sometimes exceeding 30%. Chronic exposure to such high levels of biomechanical insult would likely lead to neural tissue damage and blindness. Effects were highly variable between individuals suggesting that individual-specific characteristics are needed to predict accurately the sensitivity to the pressures and the risk to vision. Our results demonstrate the power of elastography methods based on non-invasive imaging technologies to help understand disease.

10067-12, Session 5

Quantitative analysis of retina layer elasticity based on automatic 3D segmentation

Youmin He, Yueqiao Qu, Beckman Laser Institute and Medical Clinic, Univ. of California, Irvine (United States); Yi Zhang, USC Roski Eye Institute, The Univ. of Southern California (United States); Teng Ma, Resource Ctr. for Medical Ultrasonic Transducer Technology, The Univ. of Southern California (United States); Jiang Zhu, Yusi Miao, Beckman Laser Institute and Medical Clinic, Univ. of California, Irvine (United States); Mark Humayun, USC Roski Eye Institute, The Univ. of Southern California (United States); Qifa Zhou, The Univ. of Southern California (United States); Zhongping Chen, Beckman Laser Institute and Medical Clinic, Univ. of California, Irvine (United States)

Age-related macular degeneration (AMD) is an eye condition that is considered to be one of the leading causes of blindness among people over 50. Recent studies suggest that the mechanical properties in retina layers are affected during the early onset of disease. Therefore, it is necessary to identify such changes in the individual layers of the retina so as to provide useful information for disease diagnosis. In this study, we propose using an acoustic radiation force optical coherence elastography (ARF-OCE) system to dynamically excite the porcine retina and detect the vibrational displacement with phase resolved Doppler optical coherence tomography. Due to the vibrational mechanism of the tissue response, the image quality is compromised during elastogram acquisition. In order to properly analyze the images, all signals, including the trigger and control signals for excitation, as well as detection and scanning signals, are synchronized within the OCE software and are kept consistent between frames, making it possible for easy phase unwrapping and elasticity analysis. In addition, a combination of segmentation algorithms is used to accommodate the compromised image quality. An automatic 3D segmentation method has been developed to isolate and measure the relative elasticity of every individual retinal layer. Two different segmentation schemes based on random walker and dynamic programming are implemented. The algorithm has been validated using a 3D region of the porcine retina, where individual layers have been isolated and analyzed using statistical methods. The errors compared to manual segmentation will be calculated.

10067-13, Session 5

Quantifying the effects of UV-A/riboflavin crosslinking on the elastic anisotropy and hysteresis of the porcine cornea by noncontact optical coherence elastography

Manmohan Singh, Jiasong Li, Raksha Raghunathan, Zhaolong Han, Achuth Nair, Chih-Hao Liu, Univ. of Houston

(United States); Salavat R. Aglyamov, The Univ. of Texas at Austin (United States); Michael D. Twa, The Univ. of Alabama at Birmingham (United States); Kirill V. Larin, Univ. of Houston (United States) and Baylor College of Medicine (United States)

The collagen fibril orientation of the cornea can provide critical information about cornea tissue health because diseases such as keratoconus and therapeutic interventions such as UV-A/riboflavin corneal collagen crosslinking (CXL) can alter the ultrastructural arrangement of collagen fibrils. Here, we quantify the elastic anisotropy and hysteresis of in situ porcine corneas as a function of intraocular pressure (IOP) with noncontact optical coherence elastography. Moreover, the effects of UV-A riboflavin corneal collagen crosslinking on the elastic anisotropy and hysteresis were evaluated. The propagation of an air-pulse induced elastic wave was imaged at stepped meridional angles by a home built phase-stabilized swept source OCE system. The stiffness of the cornea was translated from the velocity of the wave, and the elastic anisotropy was quantified by modifying the planar anisotropy coefficient. As the IOP increased, the stiffness of the corneas increased from ~18 kPa at 15 mmHg IOP to ~ 120 kPa at 30 mmHg IOP. While there was a measureable hysteresis, it was not significant. After CXL, the Young's modulus of the corneas significantly increased from ~18 kPa to ~44 kPa at 15 mmHg IOP. The mechanical anisotropy also increased significantly from ~10 a.u. in the untreated corneas to ~23 a.u. in the CXL treated corneas, 15 mmHg IOP. However, CXL did not change the elastic anisotropic orientation, and the mechanical anisotropic hysteresis was not significant after CXL.

10067-14, Session 5

Fluorescence spectroscopy for non-invasive measurement of mechanical stiffness after photo-crosslinking of rabbit cornea

Maura Williams, William F. Lewis, Antonio Ortega-Martinez, Walfre Franco, Massachusetts General Hospital (United States)

Keratoconus is a disease characterized by progressive steepening and thinning of the cornea, altering visual acuity and sometimes potentiating the need for corneal transplant if the disease progresses. Corneal crosslinking, a procedure that uses topical riboflavin and UV light to increase the stiffness of the cornea through the creation of collagen crosslinks was recently approved by the FDA for use in the U.S. The objective of the present study was to investigate whether endogenous collagen fluorescence changes following treatment can be correlated to alterations in the stiffness of the cornea, thereby guiding treatment parameters.

80 ex-vivo rabbit eyes were used. The epithelium was removed from each and topical riboflavin was applied. Corneas were irradiated with a 365 nm black ray UV lamp for various treatment times, ranging from half the clinical treatment time to three times as long. Mechanical testing was performed to determine the force/displacement relationship for the various treatment times. Fluorescence spectral changes following treatment corresponded with changes in stiffness. In particular, a decrease in the value of fluorescence intensity at 290/330 nm excitation/emission wavelengths corresponded to an increase in corneal stiffness following treatment. It may be possible to use fluorescence spectral changes of endogenous corneal crosslinks to evaluate mechanical stiffness changes non-invasively.

10067-15, Session 5

Assessing the changes in the spatial stiffness of the posterior sclera as a function of IOP with air-pulse OCE

Manmohan Singh, Achuth Nair, Univ. of Houston (United States); Salavat Aglyamov, The Univ. of Texas at Austin (United States); Chen Wu, Zhaolong Han, Ericka Lafon, Kirill V. Larin, Univ. of Houston (United States)

The mechanophysiology of tissues in the posterior eye have been implicated for diseases such as myopia and glaucoma. For example, the eye-globe shape, and consequently optical axial length, can be affected by scleral stiffness. In glaucoma, an elevated intraocular pressure is the primary risk factor for glaucoma, which is the 2nd most prevalent known cause of blindness. Recent work has shown that biomechanical properties of the optic nerve are critical for the onset and progression of glaucoma because weak tissues cause large displacements in the optic nerve, causing tissue damage. In this work, we utilize air-pulse optical coherence elastography (OCE) to quantify the spatial distribution of biomechanical properties of the optic nerve, its surrounding tissues, and the posterior sclera. Air-pulse measurements were made in a grid on in situ porcine eyes in the whole eye-globe configuration as various IOPs. The Young's modulus was quantified by a model-based viscoelasticity reconstruction method based on the dynamic response of the tissue to the air-pulse. The results show that the optic nerve and peripapillary sclera are much stiffer than the surrounding sclera, and the stiffness of the optic nerve and peripapillary sclera increased as a function of IOP. However, the stiffness of the surrounding sclera did not increase. Our results show that understanding the dynamics of the biomechanical properties of the eye are critical to understand the aforementioned diseases and may provide additional information for assessing visual health and integrity.

10067-16, Session 6

Rapid non-invasive mechanical imaging using line-scanning Brillouin microscopy

Jitao Zhang, Antonio Fiore, Univ. of Maryland, College Park (United States); Seok-Hyun Yun, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and Harvard-MIT Health Sciences and Technology (United States) and Harvard Medical School (United States); Hanyoup Kim, Canon U.S. Life Sciences, Inc. (United States); Giuliano Scarcelli, Univ. of Maryland, College Park (United States)

Brillouin spectroscopy is able to measure material's mechanical properties by analyzing the optical spectrum of acoustically-induced light scattering within a sample. In the past decade, the development of high-resolution Brillouin spectrometers based on virtually-imaged phased array (VIPA) has greatly increased the spectral detection efficiency thus enabling mechanical characterization of biological tissue and biomaterials. Further improvements in spectrometer performances have enabled in vivo measurements at safe power levels and 2D/3D imaging of biological cells. However, it remains a slow technique compared to other imaging modalities, because only one point of the sample can be measured by the traditional backward-scattering configuration at a time. In this work, we demonstrate a parallel detection configuration with 90-degree geometry where the Brillouin shift of hundreds of points in a line can be measured simultaneously. In a 1.1mm-by-1.5mm samples, this novel configuration effectively shortens the acquisition time of 2D Brillouin imaging from hours to ~30 seconds with spatial resolution of ~3um, thus making it a powerful technology for label-free mechanical characterization of tissue and biomaterials.

10067-17, Session 6

High-speed Brillouin profilometry of materials via continuous-wave stimulated Brillouin scattering

Itay Remer, Alberto Bilenca, Ben-Gurion Univ. of the Negev (Israel)

Brillouin spectroscopy is a noncontact technique for characterizing the mechanical properties of materials. Typically, Brillouin spectrometers have been realized using scanning Fabry–Perot spectrometers that measure, with long acquisition times, spontaneous Brillouin scattering from the samples. In the last few years, the use of virtually imaged phase array (VIPA) etalons for constructing Brillouin spectrometers has enabled to acquire spontaneous Brillouin spectra >1,000-fold faster than with scanning Fabry–Perot spectrometers, opening up new means for high-speed Brillouin analysis of materials.

In this talk, we will present a different approach for high-speed Brillouin material analysis. The method uses continuous-wave stimulated Brillouin scattering (CW-SBS) to measure stimulated Brillouin gain (SBG) spectra of materials at <100 milliseconds – up to 100-fold faster than with existing CW-SBS spectrometers. The SBS spectrometer comprises two nearly counter-propagating single-frequency lasers at 780 nm whose frequency detuning is scanned through the material Brillouin shift. SBG is detected via an ultra-narrowband hot rubidium-85 vapor notch filter and a lock-in detector, resulting in an improved signal-to-noise ratio that enables to significantly shorten acquisition times. We will show that this improvement, combined with micrometer-step-size spatial scanning of the sample, provides precise Brillouin profiles of layered liquids at 30-milliseconds pixel-dwell-time, facilitating Brillouin profilometry analysis of materials at high speed.

10067-18, Session 6

Mechanical characterization at material interfaces through dark field Brillouin microscopy

Antonio Fiore, Giuliano Scarcelli, Univ. of Maryland, College Park (United States)

Brillouin microscopy allows high–resolution mapping of the mechanical properties of a sample by measuring the spectra of acoustically induced light scattering therein, and thus has been widely investigated for biomedical application.

Measuring the Brillouin spectral shift is challenging when the light is focused onto the interfaces between two materials of different refractive index, because a sizeable portion of the incident light is Fresnel-reflected into the Brillouin spectrometer. To address this need, here, we designed a Brillouin confocal microscope in which the specular reflection at the interface between two materials is physically rejected without significant loss to the Brillouin signal.

To achieve this goal, we illuminate the sample with a small-diameter Gaussian beam focused by a high numerical aperture objective lens. In the collection path, the beam reflected from the sample has the same diameter as the incident beam, while the scattered light beam is as large as the clear aperture of the microscope objective. Therefore, using a small blocking filter allows to efficiently reject the reflected light.

We calculated the tradeoff between extinction improvement and signal loss when the diameter of the blocking filter is changed. Experimentally, we demonstrated extinction improvement of over 60dB with only 30% signal loss while achieving submicron resolutions.

This innovation can be useful for in vivo measurements of the cornea to avoid artifacts in the epithelium and anterior portions of the stroma, as well as to investigate cells cultured on glass coverslips without necessity of index-matching materials.

10067-19, Session 6

What is next to Brillouin spectroscopy in biology and medicine? *(Invited Paper)*

Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Brillouin spectroscopy is almost as old as Raman spectroscopy, but its applications to biology and medicine are lagging those of Raman spectroscopy. We believe that it is the lack or minimum availability of commercial instruments makes such advancements slow and, often, inefficient. Over the past years there appeared to be a revitalized interest in Brillouin microscopy and its biomedical applications, which triggered the development of new tools and techniques for Brillouin imaging.

In my talk, I will summarize most of the recent progress in the area of instrumentation development, which resulted in astonishing 6 orders of magnitude increase in the acquisition speed and 2 orders of magnitude improvement in spectral accuracy of Brillouin peaks' analysis. The combination of those advancements brought a succession of new applications ranging from static imaging of eye's cornea and biomaterials to dynamic changes of developing cells and tissues. At the end of my talk, I will outline several possible future directions in order to answer the question in the title of this presentation.

10067-20, Session 7

Using Brillouin microspectroscopy to characterize adipocytes' response to lipid droplet accumulation

Maria A. Troyanova-Wood, Zachary Coker, Charles Ballmann, Andrew J. Traverso, Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Obesity and overweight are accompanied by an enlargement of adipocytes, which is commonly related to the increasing number and size of lipid droplets within the cells. Some studies have shown that the accumulation of lipid droplets within adipocytes results in their increased stiffness. Recently, Brillouin microspectroscopy has been introduced as a non-destructive method of imaging the elasticity of cells. Unlike other imaging modalities, it is capable of assessing the elastic properties on both tissue- and cell levels. In this study, Brillouin spectroscopy was used to measure the changes of elasticity in response to accumulation of lipid droplets within adipocytes during adipogenesis. Supplementary Raman spectra were obtained to evaluate the ratio of lipid droplets over the entire cell volume. The results are in agreement with previous atomic force microscopy (AFM) nanoindentation studies. Brillouin microspectroscopy is a technique suitable for measuring the changes in elasticity of adipocytes in response to lipid droplet accumulation.

10067-21, Session 7

Mechanical characterisation of hydrogels using Brillouin microscopy, ultrasound and unconfined compression tests

Pei-Jung Wu, Irina V. Kabakova, ChengZe Song, Carl Paterson, Darryl R. Overby, Peter Török, Imperial College London (United Kingdom)

Mechanical characterisation of biomaterials provides the basis for investigating disease-related changes in the biomechanical properties of living tissues and cells. Brillouin microscopy offers a non-invasive and label-free method to measure material properties. Briefly, Brillouin scattering involves energy exchange between photons and acoustic phonons, resulting in an optical frequency shift of the scattered light. This shift is proportional to the speed of sound in the material, and consequently to the longitudinal

elastic modulus (M). However, it is unclear how Brillouin measurements, which characterize the mechanical response at GHz frequencies, relate to mechanical properties measured at much lower frequencies (~1 Hz) relevant to physiological conditions. Furthermore, as most biomaterials are hydrated, it remains unclear how the relative incompressibility of water influences the acoustic wave speed so as to affect Brillouin measurements of hydrated biomaterials.

In this study, we aim to establish the relationship between Brillouin frequency shift, acoustic wave speed and quasi-static elastic modulus of hydrogels of varying stiffness. Hydrogels are homogeneous and isotropic materials that mimic the poroelastic nature of biological tissues. Each measurement probes the mechanics of hydrogels in a significantly different frequency range: GHz for Brillouin imaging, MHz for ultrasound and Hz for unconfined compression tests. The acoustic wave speed falls into range from 1490 to 1533 m/s in both MHz (ultrasound) and GHz (Brillouin) frequency ranges. The quasi-static modulus correlates positively with Brillouin frequency shift, increasing from 6 to 54 kPa. All the results indicate the measurements obtained by Brillouin microscopy are capable of representing the material properties of hydrogels in quasi-static condition.

10067-22, Session 7

Selective two-photon collagen crosslinking in situ measured by Brillouin microscopy

Sheldon J. J. Kwok, Massachusetts General Hospital (United States) and Harvard Medical School (United States) and Massachusetts Institute of Technology (United States); Ivan A. Kuznetsov, Massachusetts General Hospital (United States) and Johns Hopkins Univ. (United States); Moonseok Kim, Massachusetts General Hospital (United States); Myunghwan Choi, Massachusetts General Hospital (United States) and Sungkyunkwan Univ. (Korea, Republic of); Giuliano Scarcelli, Massachusetts General Hospital (United States) and Univ. of Maryland, College Park (United States); Seok-Hyun Yun, Massachusetts General Hospital (United States) and Harvard Medical School (United States) and Massachusetts Institute of Technology (United States)

Two-photon polymerization and crosslinking are commonly used methods for microfabrication of three-dimensional structures with applications spanning from photonic microdevices, drug delivery systems, to cellular scaffolds. However, the use of two-photon processes for precise, internal modification of biological tissues has not yet been reported. One of the major challenges has been a lack of appropriate tools to monitor and characterize crosslinked regions nondestructively.

Here, we demonstrate spatially selective two-photon collagen crosslinking (2P-CXL) in intact tissue for the first time. Using riboflavin photosensitizer and femtosecond laser irradiation, we crosslinked a small volume of tissue within animal corneas. Collagen fiber orientations and photobleaching were characterized by second harmonic generation and two-photon fluorescence imaging, respectively. Using confocal Brillouin microscopy, we measured local changes in longitudinal mechanical moduli and visualized the cross-linked pattern without perturbing surrounding non-irradiated regions. 2P-CXL-induced tissue stiffening was comparable to that achieved with conventional one-photon CXL. Our results demonstrate the ability to selectively stiffen biological tissue in situ at high spatial resolution, with broad implications in ophthalmology, laser surgery, and tissue engineering.

10067-23, Session 7

Optimisation of VIPA spectrometers

ChengZe Song, Imperial College London (United Kingdom); Emilio Sánchez-Ortiga, Imperial College London (United Kingdom) and Univ. de València (Spain); Matthew R. Foreman, Peter Török, Imperial College London (United Kingdom)

VIPA (Virtually Imaged Phased Array) based spectrometers are now routinely favoured over other types of spectrometers (such as scanning Fabry-Perot) for Brillouin imaging because VIPAs permit higher data acquisition speeds as compared to others. However, higher speeds mean lower photon counts at the camera used to acquire the spectra. The quality of optical components used is also important and have profound effect on the quality of the spectrum. Yet, these issues have not been addressed by various groups doing Brillouin imaging around the world. In this talk we examine the effect of the various optical components on the overall performance of the spectrometer both in one and two stage configuration. We define information content in the measured spectra and using information theoretic approach determine system parameters under various design conditions. We show for example, the spherical aberration imparted by the plano-convex cylinder lens usually placed at the entrance of the spectrometer reduces signal quality but it otherwise does not affect the accuracy of measurements. On the other hand, aberrations introduced by lenses further down the optical train may result in significant loss in localisation accuracy of the spectra. Our approach will aid users of VIPA based spectrometers designing better quality systems.

10067-24, Session 7

Brillouin fibre endoscope for stiffness measurements in biological tissues

Yuchen Xiang, ChengZe Song, Imperial College London (United Kingdom); William J. Wadsworth, Univ. of Bath (United Kingdom); Carl Paterson, Peter Török, Irina V. Kabakova, Imperial College London (United Kingdom)

Brillouin imaging has recently emerged as a powerful technique for its ability to give insight to the mechanical properties of biomaterial. It exploits inelastic scattering of light by acoustic vibrations and maps the tissue stiffness point by point with micron resolution. The non-invasive, real-time nature of the measurements also makes it a potent candidate for in-vivo imaging of live cells and tissues. This, however, has to rely on a compact and flexible apparatus, a Brillouin endoscope, for remote access to specimen parts.

One of the main challenges encountered in the construction of Brillouin endoscope is that the inelastic scattering in the fibre conduit itself is orders of magnitude stronger than the Brillouin signal scattered by the specimen. This is because the length of the fibre endoscope (meters) is orders of magnitude larger than the imaging volume (microns). The problem can be overcome if the scattered light is collected by a separate fibre and does not mix with the fibre scattering inside the delivery channel.

Here we present an all-fibre integrated Brillouin microspectroscopy system that exploits the paths separation between delivery and collection channels. The experimental setup consists of a pair of standard silica single-mode fibres coupled to a graded-index lens and illuminated with a 671nm continuum wavelength source. We test our system performance on liquid samples of water and ethanol and confirm Brillouin shifts of 5.9 GHz and 4.6 GHz, respectively. More importantly, we do not observe any signals corresponding to Brillouin shift in the fibre, in agreement with expectation.

10067-25, Session 8

Investigation of stress-induced birefringence of tissue determined with polarisation sensitive optical coherence tomography

Karol Karnowski, Qingyun Li, The Univ. of Western

Australia (Australia); Martin Villiger, Harvard Medical School (United States) and Massachusetts General Hospital (United States); David D. Sampson, The Univ. of Western Australia (Australia)

Polarisation sensitive optical coherence tomography (PS-OCT) offers additional intrinsic contrast to probe differences between healthy tissue and cancer that are often barely visible due to limited scattering contrast in an OCT image. PS-OCT reconstructs tissue birefringence from phase-sensitive measurements of orthogonal polarisation components of backscattering. In material science, polarisation has been used to study stress distribution, including the birefringence induced by stress in an otherwise isotropic material. Similar effects in biological tissues have not been well studied yet; however, may have application to tissues subjected to stress, e.g., tendons, muscles, lens, cornea or airway smooth muscle (ASM). The objective of this work is to explore stress-induced birefringence in tissue. We employ an advanced swept source-based PS-OCT system capable of measurement of tissue local polarisation properties. The sample in both cases is illuminated with orthogonal, passively depth-encoded polarisation states. Light returning from the tissue is detected via a polarisation-diversity detection module and a Mueller formalism is used to reconstruct polarisation properties (including retardation, diattenuation, and depolarisation) of the tissue. In this study, we demonstrate the measurement of stress-induced birefringence in phantoms and in soft tissues with polarisation sensitive optical coherence tomography.

10067-26, Session 8

Investigating mechanically induced phase response of the tissue by using high-speed phase-resolved optical coherence tomography

Yuye Ling, Christine P. Hendon, Columbia Univ. (United States)

Phase-resolved optical coherence tomography (OCT), a functional extension of OCT, provides depth-resolved phase information with extra contrast. In cardiology, changes in the mechanical properties have been associated with tissue remodeling and disease progression. Here we present the capability of profiling structural deformation of the sample in vivo by using a highly stable swept source OCT system The system, operating at 1300 nm, has an A-line acquisition rate of 200 kHz. We measured the phase noise floor to be 6.5 pm±3.2 pm by placing a cover slip in the sample arm, while blocking the reference arm. We then conducted a vibrational frequency test by measuring the phase response from a polymer membrane stimulated by a pure tone acoustic wave from 10 kHz to 80 kHz. The measured frequency response agreed with the known stimulation frequency with an error < 0.005%. We further measured the phase response of 7 fresh swine hearts obtained from Green Village Packing Company through a mechanical stretching test, within 24 hours of sacrifice. The heart tissue was cut into a 1 mm slices and fixed on two motorized stages. We acquired 100,000 consecutive M-scans, while the sample is stretched at a constant velocity of 10 um/s. The depth-resolved phase image presents linear phase response over time at each depth, but the slope varies among tissue types. Our future work includes refining our experiment protocol to quantitatively measured the elastic modulus of the tissue in vivo and building a tissue classifier based on depth-resolved phase information.

10067-27, Session 8

Quantitative analysis of a scar's pliability, perfusion and metrology

Mariacarla Gonzalez, Susan Stoff, Joseph Chue-Sang, Nicole Sevilla, Jessica C. Ramella-Roman, Florida International Univ. (United States)

Secondary effects of scarring includes the loss of sweat glands, development of cancer in scar tissue and skin neurosensory malfunctions, therefore accurate evaluation is necessary. Currently, scar assessment is highly subjective and physician dependent. The examination relies on the expertise of the physician to determine the characteristics of the scar by touch and visual examination using the Vancouver scar scale (VSS), which categorizes scars depending on pigmentation, pliability, height and vascularity. In order to establish diagnostic guidelines for scar formation, a quantitative, accurate assessment method needs to be developed. We will focus on the diagnosis of pliability, perfusion and metrology for scar evaluation.

An instrument capable of measuring all three modalities was developed. The system consists of a durometer, a laser speckle imaging system all integrated with a commercial profilometer (FaroArm). A durometer measures the amount of resistance a surface exerts to prevent the permanent indentation of the surface; it is simple to use and provides a quantitative metric. The FaroArm evaluates the location of the scar in three-dimensions and can provide scar-size and height. Furthermore, the extent of damage to the skin can be perceived in the vascularity of the scar. Using laser speckle perfusion imaging (LSPI), the dynamic changes in blood-flow can be observed. Gelatin phantoms are utilized to measure pliability and location, which allow for quantitative comparison to the existing scale. Dynamic changes in skin perfusion were measured in volunteers' forearms undergoing pressure cuff occlusion. Finally, incisional scars of human volunteers were measured with this new device.

10067-28, Session 8

Inverse methods for three dimensional quantitative optical coherence elasticity imaging

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In elastography, quantitative elastograms are desirable as they are system and operator independent. Such quantification also facilitates more accurate diagnosis, longitudinal studies and studies performed across multiple sites. In optical elastography (compression, surface-wave or shear-wave), quantitative elastograms are typically obtained by assuming some form of homogeneity. This simplifies data processing at the expense of smearing sharp transitions in elastic properties, and/or introducing artifacts in these regions.

Recently, we proposed an inverse problem-based approach to compression OCE that does not assume homogeneity, and overcomes the drawbacks described above. In this approach, the difference between the measured and predicted displacement field is minimized by seeking the optimal distribution of elastic parameters. The predicted displacements and recovered elastic parameters together satisfy the constraint of the equations of equilibrium. This approach, which has been applied in two spatial dimensions assuming plane strain, has yielded accurate material property distributions.

Here, we describe the extension of the inverse problem approach to three dimensions. In addition to the advantage of visualizing elastic properties in three dimensions, this extension eliminates the plane strain assumption and is therefore closer to the true physical state. It does, however, incur greater computational costs. We address this challenge through a modified adjoint problem, spatially adaptive grid resolution, and three-dimensional decomposition techniques. Through these techniques the inverse problem is

solved on a typical desktop machine within a wall clock time of ~ 20 hours. We present the details of the method and quantitative elasticity images of phantoms and tissue samples.

10067-29, Session 9

Utilising non-linear elasticity to increase mechanical contrast in quantitative optical coherence elastography

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Compression optical coherence elastography (OCE) enables rapid acquisition with high resolution over fields of view relevant to many clinical applications. Compression OCE typically provides a relative measure of mechanical properties; however, we have recently demonstrated a technique which quantifies stiffness via a compliant layer, termed quantitative OCE. In quantitative OCE, stiffness is reported as a tangent modulus, which is a surrogate for Young's modulus at a given preload in non-linear elastic material. In biological tissues, which are typically non-linear elastic, values of stiffness reported through quantitative OCE could be over- or under-estimated, and are heavily biased by the arbitrary bulk preload applied to that region.

We present a method to measure tissue nonlinearity locally, by preforming compression OCE at multiple preloads ranging from 2% to 40%. We show, through presentation of 2D quantitative elastograms, that compression OCE has the potential to measure the non-linear stiffness in tissue mimicking phantoms and biological tissue. Further, intrinsic mechanical contrast in tissue is dependent upon its preload. By tailoring tissue preload, we demonstrate improved contrast between benign and tumor tissue in a murine liver carcinoma model.

10067-30, Session 9

Computationally-efficient optical coherence elastography to quantitatively assess degenerative osteoarthritis with high acoustic wave compressive load

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We demonstrate a computationally-efficient optical coherence elastography (OCE) method based on fringe washout. By introducing ultrasound in alternating depth profile, we can obtain information on the mechanical properties of a sample within acquisition of a single image. This can be achieved by simply comparing the intensity in adjacent depth profiles in order to quantify the degree of fringe washout. Phantom agar samples with various densities were measured and quantified by our OCE technique, the correlation to Young's modulus measurement by atomic force micrscopy (AFM) were observed. Knee cartilage samples of monoiodo acetate-induced arthiritis (MIA) rat models were utilized to replicate cartilage damages where our proposed OCE technique along with intensity and birefringence analyses and AFM measurements were applied. The results indicate that our OCE technique shows a correlation to the techniques as polarization-sensitive OCT, AFM Young's modulus measurements and histology were promising. Our OCE is applicable to any of existing OCT systems and demonstrated to be computationally-efficient.

10067-31, Session 9

Multiparameter thermo-mechanical OCT-based characterization of laser-induced cornea reshaping

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Phase-sensitive optical coherence tomography (OCT) is used for visualizing dynamic and cumulative strains and cornea-shape changes during laser-produced tissue heating. Such non-destructive (non-ablative) cornea reshaping can be used as a basis of emerging technologies of laser vision correction. We apply an original phase-processing approach for estimating thermally-induced strain fields. The used processing obviates conventional procedures of phase unwrapping and allows for visualization of large laser-induced cumulated strains based on interframe comparison. Complementary estimates of both reversible thermal expansion and accumulated plastic-type deformations can be combined with heating temperature estimates via OCT-based measurements of variations in thermal expansion coefficient. We demonstrate temporal plastification of cornea without affecting its transparency allowing for its reshaping sufficient for vision-correction applications. In ex-vivo experiments with excised rabbit eyes we demonstrate ability of the developed OCT system to simultaneously characterize transient and cumulated strain distributions, surface displacements, scattering tissue properties and possibility of temperature estimation via thermal-expansion measurements. The method allows for reconstructing strains even for supra-wavelength interframe displacement of particles, whereas fairly sparse data acquisition is sufficient. Experimental results are compared with findings of previously used methods for studying laser-induced reshaping of cartilaginous tissues, numerical simulations, as well as literature data on OCT methods used for studying laser coagulation in eye retina. The proposed approach can be implemented in perspective real-time OCT systems for controlling laser-induced cornea deformation with 10-100 ms time resolution, combined with possibility of estimating attained temperature and sufficiently long-term monitoring for ensuring safety of new methods of laser reshaping of cornea.

10067-32, Session 9

Non-invasive structural and biomechanical imaging of the developing embryos using OCT and Brillouin microscopy

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Embryos undergo dramatic changes in size, shape, and mechanical properties during development, which is regulated by both genetic and environmental factors. Quantifying mechanical properties of different embryonic tissues may represent good metrics for the embryonic health and proper development. Alternations and structure coupled with biomechanical information may provide a way for early diagnosis and drug treatment of various congenital diseases. Many methods have been developed to determine the mechanical properties of the embryo, such as atomic force microscopy (AFM), ultrasound elastography (UE), and optical coherent elastography (OCE). However, AFM is invasive and time-consuming. While UE and OCE are both non-invasive methods, the spatial resolutions are limited to mm to sub-mm, which is not enough to observe the details inside the embryo. Brillouin microscopy can potentially enable non-invasive measurement of the mechanical properties of a sample by measuring the spectra of acoustically induced light scattering therein. It has fast speed (~0.1 second per point) and high resolution (sub-micron), and thus has been widely investigated for biomedical application, such as single cell and tissue. In this work, we utilized this technique to characterize the mechanical property of an embryo. A 2D elasticity imaging of the whole body of an E8 embryo was acquired by a Brillouin microscopy, and the stiffness changes between different organs (such as brain, heart, and spine) were shown. The elasticity maps were correlated with structural information provided by OCT.

10067-38, Session PSun

Improved phase stability and detection sensitivity in sample-arm-interference optical coherence elastography (SAI-OCE)

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Phase-resolved optical coherence elastography (OCE) was developed to quantify tissue mechanical properties (e.g. stiffness). Environmental turbulence (e.g. vibration, temperature change, air flow, etc.) exists between sample and reference arms in OCE and is a major source of dynamic background phase noise. To address this problem, we recently developed sample-arm-interference OCE (SAI-OCE), in which the interference signal is produced by combing scattered or reflected light from the sample and a reference plane adjacent to the sample. Optical phase stability for SAI-OCE and conventional OCE were compared in mirror measurements. Likewise, the precision of dynamic surface displacement measurements for each method was evaluated using agar tissue phantoms. The dominant component of the phase noise in conventional OCE was low frequency (20.80±1.23 Hz) and amplitude (3.69±0.71 radians [250±47.5nm]). The dominant phase noise in SAI-OCE had similar frequencies (26.20±3.88 Hz), but much lower amplitude (4.00±1.35 milliradians [0.27±0.09nm]). The average standard deviation values for displacement measurement were 0.955 radians [64.2nm] in conventional OCE and 0.233 radians [15.7nm] in SAI-OCE. The SAI technique effectively reduces the amplitude of background dynamic optical phase instability ~1000 times lower than conventional phase-sensitive OCE to a sub-nanometer level. SAI-OCE can also provide a 4-fold improvement in the precision of displacement measurements. SAI-OCE is capable of providing a more stable baseline from which to measure surface displacement. This can improve quantification of dynamic phenomena during elastography imaging.

10067-39, Session PSun

Optimization of dental implantation

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Modern dentistry cannot exist without dental implantation. The lifetime of the installed implants depends on condition of the bone and on the quality of the treatment planning and surgery technique. Usually, complications during the implant treatment are related to the inability to accurately predict the condition and location of intraosseous structure that entails the selection of the wrong type of implant and installation position.

This work is devoted to the "bone-implant" system investigation aiming on the optimization of dental prostheses installation. The objective of this study was to develop the implant treatment planning technique. Modern non-invasive methods such as computer tomography (CT) and 3D-scanning as well as numerical calculations and 3D-prototyping allow optimizing all of dental prosthetics stages.

In this work, complex methodology of dental prosthesis installation is presented. Patient-specific dental templates developed in this work are easy to create and very accurate.

CT processing method, numerical modeling and 3D prototyping techniques allowed us to develop a methodology of dental prosthesis installation. An integrated approach to the planning of implant surgery can significantly reduce the risk of complications in the first few days after treatment, and throughout the period of operation of the prosthesis.

10067-40, Session PSun

Evaluation of dermal fillers with noncontact optical coherence elastography

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Over 2 million dermal filler procedures are performed each year in the USA alone, and this figure is only expected to increase as the aging population continues to grow. Dermal filler treatments can last from a few months to years depending on the type of filler and its placement. Although adverse reactions are rare, they can be quite severe due to ischemic events and filler migration. Previously, techniques such as ultrasound or magnetic resonance imaging have been used to evaluate the filler injections. However, these techniques are not practical for real-time filler injection guidance due to limitations such as the physical presence of the transducer. In this work, we propose the use of optical coherence tomography (OCT) for image-guided dermal filler injections due to the high spatial and temporal resolution of OCT. In addition, we utilize a noncontact optical coherence elastography (OCE) technique, to evaluate the efficacy of the dermal filler injection. A grid of air-pulse OCE measurements was taken, and the dynamic response of the skin to the air-pulse was translated to the Young's modulus and shear viscosity. Our results show that OCT was able to visualize the dermal filler injection process, and that OCE was able to localize the dermal filler injection sites. Combined with functional techniques such as optical microangiography, and recent advanced in OCT hardware, OCT may be able to provide real-time injection guidance in 3D by visualizing blood vessels to prevent ischemic events.

10067-41, Session PSun

The strength of bones: from molecular to macroscopic scale sensing using micro-Brillouin light scattering spectroscopy

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We evaluated biomechanical properties of bones using non-invasive Brillouin light scattering spectroscopy. Bones serve as a load-bearing framework for a body. Biomechanical properties of bones, such as

toughness and plasticity, are essential for understanding how microscopic scale mechanical features can link to macroscale bones' strength and fracture resistance.

In the reported work, collagen fibers harvested from different tissues and cortical bones of different mammalian species were used. Fresh cut samples of bovine flexor tendon and collagen fibers from the rat's tails were cut along and across of the fiber axis. Tibia bones of sheep, chicken and turkey were cleaned and cut into small pieces along radial plane and were investigated under different compressive loads applied along the bones' axial direction. We used a commercially available Brillouin spectrometer based on a 6-pass scanning tandem Fabry-Perot interferometer in a confocal arrangement.

Experimental results exhibited strong mechanical anisotropy of collagen tissue samples measured across and along the cut of the tendon. All bones' samples revealed an increase of Young's modulus with the increase of applied load until reaching some critical point.

Brillouin light scattering has proven to be a powerful non-contact, non-invasive, and label-free technique capable of assessing biomechanical properties at different scales for a variety of biomedical applications. In our conference presentation we will discuss our new results on Brillouin microspectroscopy of bones and its constituents in terms of the structure-property relationship and the related biological function.

10067-42, Session PSun

Mechanical heterogeneity: facilitating rapid interpretation of human breast elastograms

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Breast-conserving surgery is a common procedure for treating breast cancer, with the aim, in a single operation, of removing a minimum of tissue, whilst ensuring that the surgical margin is free of cancer. Intraoperative assessment of tumor margins is, however, not exact; thus, re-excision is frequently needed, or excess normal tissue is removed.

Optical coherence elastography (OCE) has been proposed for use in breast-conserving surgery; however, intraoperative interpretation of often complex OCE images (elastograms) may prove challenging. Assessment of breast cancer on multiple length scales, by atomic force microscopy, OCE, and ultrasound elastography, have shown an increase in the mechanical heterogeneity of malignant breast tumors compared to normal breast tissue. Measurement and mapping of this heterogeneity may enable simplified, and hence rapid, interpretation of elastograms. This could aid in the real time identification of regions of possible malignancy during surgery.

In this study, we introduce a heterogeneity index, inversely proportional to the correlation length (the distance at which the autocorrelation falls to zero) of the tissue strain over local regions. Calculating this index over en face elastograms, we form maps of the mechanical heterogeneity of samples. Through comparison with OCE, optical coherence tomography, and corresponding histology of human breast tissue samples, we show the association between malignant/benign tissue and high/low heterogeneity. The heterogeneity map simplifies the contrast in elastograms, facilitating the rapid identification of possible areas of malignancy. Our results suggest that this technique could hold promise for providing enhanced guidance of tumor margin status to clinicians during surgery.

10067-43, Session PSun

Temperature-dependent protein denaturation and coagulation monitoring using speckle variance optical coherence tomography

Changho Lee, Gyeong Woo Cheon, Jin U. Kang, Johns Hopkins Univ. (United States)

Proteins are crucial compositions of all living things for generating biological components as well as maintaining all life activities. Proteins naturally remains as stable three-dimensional structures with their specific functions. When stable structured proteins are influenced by external stresses such as acid, radiation, and heat, their original structural formation are changed. These deformations such as denaturation cause alteration of physical characteristics and activities of biological tissues. The ability to detect and monitor the process of proteins' denaturation and coagulation will be critical to many thermal therapies. In this study, we demonstrated the feasibility of using speckle variance optical coherence tomography (SvOCT) for monitoring thermal protein denaturation and coagulation process. By providing micro-scaled depth-revealed optical images, SvOCT can be used to detect and monitor the process of proteins denaturation and coagulation. In this work, we used an in-house-built SvOCT to study temperature-dependent denaturation and coagulation. SvOCT not only offered B-scan SvOCT images that show changes in sample morphology , it also allows denaturation quantification through speckle variances value and the cross-correlation coefficient g. Based on these information, the states of denaturation was successfully estimated.

10067-44, Session PSun

Optimal selection of laser modulation parameters in photothermal optical coherence tomography

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Photothermal optical coherence tomography (PT-OCT) is an extension of conventional OCT with ability to perform absorption imaging. In PT-OCT, a secondary intensity-modulated photothermal laser causes thermal strains leading to modulation of the refractive index in the proximity of absorbing chromophores. These variations are directly detected with phase-sensitive OCT and offer insight to the thermo-elastic properties of the sample. To date, PT-OCT has been primarily modeled and performed in absorbing bulk-media, ignoring the diffusion of the generated thermal waves. Here, we investigate the effect of the photothermal laser power and modulation frequency on the ensuing thermal waves and their impact on spatial resolution of PT imaging, using scattering phantoms made from agarose and intralipid, which contain spatially structured absorbing inclusions of varying size and geometry. Key components of our spectral-domain system include a broadband superluminescent diode centered at 1310nm (+/- 75 nm at 10dB), a 2048-pixel line scan camera spectrometer with a maximum acquisition rate of 147 kHz, and a 808nm intensity-modulated photothermal laser. At low PT modulation frequencies, strong thermal waves with large thermal diffusion lengths are generated, which yield prominent signatures in the OCT phase signal, but come at the cost of inferior imaging resolution due to the interference of thermal waves. On the other hand, increasing the PT laser modulation frequency significantly reduces the diffusion length of thermal waves, minimizing their interference and yielding better PT-OCT imaging resolution, yet limiting the phase modulation. Balancing between these two opposing mechanisms, we define criteria for optimal PT laser modulation parameters.

10067-45, Session PSun

Automated fiber tracking in the anterior cruciate ligament

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Collagen fiber organization is an important architectural feature that affects the mechanical function of many organ systems. We present an automated algorithm to quantify fiber organization within the anterior cruciate ligament (ACL). Images were collected from both the ligament proper and the ligament-bone interface of juvenile bovine ACL samples (n=8) using two spectral domain OCT systems, Thorlabs Telesto1 with 6.5 μm axial and 15 μm lateral resolution and a custom high resolution system with 2.7 μm axial and 5.52 μm lateral resolutions. Edge detection and median filtering were employed to determine ligament fiber angles. The edge detection convolution vector is aligned parallel to the fibers. The fiber spatial frequency and directionality is different than the inherent crimping pattern in this tissue type, which distinguishes the fibers from the crimping pattern. Automated segmentation of the ligament-bone interface to ascertain insertion angles was employed using entropy filtering followed by edge detection. Following morphological processing, the longest continuous segment is selected to be the interface. Based on the segment morphology, algorithm performance can be determined. Evaluating the performance within volumes containing only ligament tissue was used to validate the algorithm, where fibers within the ligament portion should be parallel to each other. The fiber-tracking algorithm verifies this, with deviations of the angles being around 15° (n=3). Insertion angles are around 30 to 40° (n=3), with a deviation of approximately 8°. Our next steps include comparing the change in insertion angle with age and full volume analysis of fiber directionality.

10067-46, Session PSun

Assessing the viscoelasticity of chicken liver by OCE and a Rayleigh wave model

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This study investigates the feasibility of assessing the viscoelasticity of soft tissues by noncontact optical coherence elastography (OCE) technique coupled with a Rayleigh wave model. An air-pulse induced elastic wave was measured by OCE. Spectral analysis obtained the dispersion curve, which was fitted to an analytical model of the Rayleigh wave model to determine the Young's modulus and viscosity of samples. In order to validate the method, 10% gelatin phantoms with and without oil were prepared and tested by OCE and mechanical testing. Results demonstrated that the elasticity ranges as assessed by the Rayleigh wave model generally agreed well with mechanical testing, and that the viscosity in the phantom with oil samples was higher than of those without oil, which was also in agreement with the literature. Further, this method was applied to quantify the viscoelasticity of chicken liver. The Young's modulus was E=2.04±0.88 kPa and the shear viscosity was ?=1.20±0.13 Pa?s with R2=0.96±0.04 between the OCE-measured dispersion curve and Rayleigh wave analytical model. Combining OCE and the Rayleigh wave model shows promise as an effective tool for noninvasively quantifying the viscoelasticity of soft tissues.

10067-47, Session PSun

Characteristics of blood components: markers of diseases as assessed by optical techniques

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This paper focuses at the characteristics of blood that can be measured in-vitro and/or in-vivo by laser-optic techniques, and which alterations can be considered as an indication of disease. The techniques to be discussed are: laser diffractometry of erythrocytes, laser scattering aggregometry of erythrocytes, digital optical capillaroscopy and measurement of the microcirculation parameters, erythrocytes trapping and manipulation with laser tweezers, fluorescence spectroscopy of blood plasma, etc. Experimental protocols and results of measurements performed in-vitro with the samples of whole human and rat blood and blood components will be outlined. Blood for the experiments in-vitro was drawn from clinically healthy human volunteers (control) and from patients suffering from diabetes mellitus, hypertension and other diseases. In-vivo measurements of the microcirculation parameters were conducted with control human subjects and patients suffering hypertension. For the in-vitro experiments with rat blood, the samples were drawn from healthy (control) and sham operated animals, those with experimentally induced diabetes mellitus and/or hypertension. Samples of human and rat blood plasma separated from blood cells were used to study the aggregation of plasma proteins that leads to the loss of their functional properties and is one of the causes of socially important diseases. Fluorescence spectroscopy allows also for identifying other alterations at the molecular level in blood plasma caused by proteins conformational changes that may induce pathological changes at the cellular level and so on up to the level of the whole organism.

The obtained results allow us to conclude that the overviewed laser-optic techniques comprise a powerful tool for efficiently identifying and assessing a set of clinically informative bio-optical markers of diseases.

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10067-48, Session PSun

Biomechanical imaging of subcellular structures by Brillouin microscopy

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Spontaneous Brillouin scattering is an inelastic scattering process arising from inherent thermal density fluctuations, or acoustic phonons, propagating in a medium. The recent development of high throughput efficiency Virtually Imaged Phased Array (VIPA) etalons and high sensitivity CCD cameras has dramatically reduced the data acquisition time, in turn enabling the extension of Brillouin spectroscopy from a point sampling technique to an imaging modality. Hitherto Brillouin microscopy has shown great capabilities to non-invasively assess the biomechanics in the volume of biological samples, such as the lens cornea, atherosclerotic plaques and cells.

In this work, Brillouin microscopy was validated in a controlled setting to investigate the subcellular biomechanical properties in healthy primary cells in vitro. The data presented here indicate that separate cellular compartments such as the cytoplasm, nuclear membrane, and nucleoli have markedly different mechanical properties. In addition, cytoplasmic stiffness was significantly reduced after administration of the drug latrunculin-A. In contrast, nucleoli did not exhibit significant changes in stiffness in response to latrunculin-A. These observations are consistent with our hypotheses because latrunculin-A acts by preventing polymerisation of the actin cytoskeleton, a protein that is omnipresent in the cytoplasm but is almost absent from the nucleus and nucleoli. As such, these results validate Brillouin microscopy as a technique to investigate the cellular and

subcellular mechanical properties of a volume of cells in vitro, and their changes over time or in response to external stimuli.

10067-33, Session 10

Three-dimensional rapid visualization of matrix deformations around angiogenic sprouts

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At the cell - extracellular matrix interface, physiologically important traction forces exerted by angiogenic sprouts can be investigated indirectly by mapping the consecutive matrix deformations. In this paper we present an approach to study these forces in three dimensions and with high time resolution. The technique employs lightsheet microscopy, in which a sheet of light is used to illuminate the sample - resulting in z-sectioning capability, superior image recording speed and reduced phototoxicity.

For this study, human umbilical vein endothelial cells (HUVEC) are transduced with a LifeAct adenoviral vector to visualize the actin cytoskeleton during live sprouting into a collagen type I hydrogel. The calculation of the matrix deformations is formulated as a B-spline-based 3D non-rigid image registration process that warps the image of beads inside the stressed gel to match the image after stress relaxation.

Using this approach we study the role of fast moving actin filaments for filopodia- and tip-cell dynamics in 3D under chemically defined culture conditions such as inhibited acto-myosin force generation. With a time resolution in the range of ten seconds, we find that our technique is at least 20 times faster than conventional traction force microscopy based on confocal imaging. Ultimately, this approach will shed light on rapid mechano-chemical feedback mechanisms important for sprouting angiogenesis.

10067-34, Session 10

Real-time and non-invasive measurements of cell mechanical behaviour with optical coherence phase microscopy

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BACKGROUND: There is an unmet need in tissue engineering for non-invasive, label-free monitoring of cell mechanical behaviour in their physiological environment. We demonstrate that optical coherence phase microscopy (OCPM) can map relative cell mechanical properties in monolayers and 3D systems under cyclic stress.

METHODS: An OCPM system was developed around a commercial spectrometer. The custom scanning head can operate in an ad-hoc mode allowing the collection of varying phase over time, and in depth into the sample, with increased phase stability .

Cyclic stress was applied non-destructively and in a contactless way to breast cancer cells (MCF-7) and mouse fibroblasts (3T3) within a microfluidic chip by a microfluidic pump.

A 4D data cube was captured with a frequency of 1.2kHz to sample varying phase over time which was converted to displacement with a custom designed set of algorithms involving the experimentally measured phase root mean square.

RESULTS: Cyclic stress was successfully applied directly to cells and the corresponding displacement was recorded in real-time at the nanometre scale for each pixel of the cell. A change in amplitude and/or frequency of the stimuli was translated to a corresponding cell response. Differences were observed in relative strain rates between the cell lines under investigation.

DISCUSSION & CONCLUSIONS: We have described a new method to monitor cell response to cyclic hydrostatic pressure in real-time and non-destructively. This can be directly related to the biomechanical properties of cells.

10067-35, Session 10

Elastic resonator interference stress microscopy (ERISM): A new tool for the long-term measurements of cellular forces

Nils M. Kronenberg, Philipp Liehm, Malte C. Gather, Univ. of St. Andrews (United Kingdom)

Mechanical forces at the cellular level are increasingly recognized as an important factor in numerous biological processes. Here, we present Elastic Resonator Interference Stress Microscopy (ERISM) as a fundamentally new approach to measure cellular forces.

ERISM is based on interferometrically detecting deformations of an elastic micro-cavity which acts as substrate for the cells. This enables fast displacement mapping – if required even online – and offers a large dynamic range and nm-accuracy (sub-Pa stress resolution). We will show how ERISM overcomes many limitations of existing methods like TFM. For instance, the micro-cavities used for ERISM have excellent long-term stability and the optical readout avoids phototoxic effects. This allows us to continuously monitor cellular forces, even during processes that last weeks. Cellular stress maps can be recorded without recording zero-stress images, which eliminates the need to detach cells after investigation (thus enabling immunostaining) and allows measurements of multiple cells on one substrate.

We have employed ERISM in several studies of cell mechanics and found it to be very widely wide applicable. Here, we will illustrate in particular the long-term time-lapse capability of ERISM by showing data on cell migration and cell differentiation. We also investigate minute vertical stresses exerted by amoebae migrating through spatial confinement and by leukocytes during a podosome-assisted form of migration. Finally, the prospect of using the high sensitivity of ERISM to measure intracellular protein interaction will be discussed and an atomic force microscope based quantitative calibration of the force sensitivity and spatial resolution of ERISM will be presented.

10067-36, Session 11

Observation of skull-guided acoustic waves in a water-immersed murine skull using optoacoustic excitation

Héctor Andrés Estrada Beltrán, Helmholtz Zentrum München GmbH (Germany); Johannes Rebling, Daniel Razansky, Helmholtz Zentrum München GmbH (Germany) and Technische Univ. München (Germany)

The skull bone, a curved solid multilayered plate protecting the brain, constitutes a big challenge for the use of ultrasound-mediated techniques in neuroscience. Ultrasound waves incident from water or soft biological tissue are mostly reflected when impinging on the skull. To this end, skull properties have been characterized for both high intensity focused ultrasound (HIFU) operating in the narrowband far-field regime and optoacoustic imaging applications. Yet, no study has been conducted to characterize the near-field properties of water immersed skulls.

We used the thermoelastic effect with a 532 nm pulsed laser to trigger a wide range of broad-band ultrasound modes in a mouse skull. In order to capture the waves propagating in the near-field, a thin hydrophone was scanned in close proximity to the skull's surface. While Leaky pseudo-Lamb waves and grazing-angle bulk water waves are clearly visible in the

spatio-temporal data, we were only able to identify skull-guided acoustic waves after dispersion analysis in the wavenumber-frequency space. The experimental data was found to be in a reasonable agreement with a flat multilayered plate model.

10067-37, Session 11

Decorrelation-based viscosity measurement using phase-sensitive optical coherence tomography

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A robust method to measure viscosity of microquantities of biological samples, such as blood and mucus, could lead to a better understanding and diagnosis of diseases. Microsamples have presented persistent challenges to conventional rheology, which requires bulk quantities of a sample. Alternatively, fluid viscosity can be probed by monitoring microscale motion of particles. Here, we present a decorrelation-based method using M-mode phase-sensitive optical coherence tomography (OCT) to measure particle Brownian motion. This is similar to previous methods using laser speckle decorrelation but with sensitivity to nanometer-scale displacement. This allows for the measurement of decorrelation in less than 1 millisecond and significantly decreases sensitivity to bulk motion, thereby potentially enabling in vivo and in situ applications. From first principles, an analytical method is established using M-mode images obtained from a 47 kHz spectral-domain OCT system. A g(1) first-order autocorrelation is calculated from windows containing several pixels over a time frame of 200-1000 microseconds. Total imaging time is 500 milliseconds for averaging purposes. The autocorrelation coefficient over this short time frame decreases linearly and at a rate proportional to the diffusion constant of the particles, allowing viscosity to be calculated. In verification experiments using phantoms of microbeads in 200 μL glycerol-water mixtures, this method showed insensitivity to 2 mm/s lateral bulk motion and accurate viscosity measurements over a depth of 400 μm. In addition, the method measured a significant decrease of the apparent diffusion constant of soft tissue after formalin fixation, suggesting potential applications in mapping tissue stiffness.

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10068-1, Session 1

Fast quantitative retardance imaging of biological tissues using quantitative phase imaging

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We describe a technique based on the use of a high-resolution quadri-wave lateral shearing interferometer to perform quantitative linear birefringence measurements on biological samples [1] such as living cells and tissues. The system combines QPI with different excitation polarizations to create retardance images. This creates a new kind of image contrast based on the local retardance, reveals the structure of sample anisotropic components and adds specificity to label-free phase images. We implemented this technique allowing us to take retardance images in less than 1 second which allows us to make high speed acquisitions to reconstruct tissues virtual slides with different modalities (i.e intensity, phase and retardance). Comparisons between healthy and tumoral 10 μm thick skin tissues and collagen orientation studies in the latter will be presented.

[1] S. Aknoun, P. Bon, J. Savatier, B. Wattellier, and S. Monneret, "Quantitative retardance imaging of biological samples using quadriwave lateral shearing interferometry," Opt. Express 23, 16383-16406 (2015).

10068-2, Session 1

Highly multiplexed IHC in clinical tissue biopsies using multiplexed ion beam imaging *(Invited Paper)*

Michael Angelo M.D., Stanford School of Medicine (United States)

Multiplexed ion beam imaging (MIBI) is a novel approach to immunohistochemistry (IHC) that uses secondary ion mass spectrometry (SIMS) and antibodies labeled with elemental mass tags to visualize dozens of proteins simultaneously in a single tissue section. MIBI is compatible with formalin-fixed, paraffin-embedded (FFPE) tissue specimens and can achieve single molecule sensitivity across a five log dynamic range. To permit broader use, we have constructed a novel imaging mass spectrometer capable of super resolution imaging and 100-fold faster sample throughput than previously reported. These tools are being used to comprehensively enumerate immune cell populations in normal and neoplastic solid tissues, to construct classifiers for predicting disease progression in pre-invasive cancer lesions, and to develop quantitative IHC assays to be used in a clinical setting.

10068-3, Session 1

New mononuclear leukocyte-like populations within the granulocyte scatter gate detected by flow cytometry *(Invited Paper)*

Susanne Melzer, Markus Löffler, Univ. Leipzig (Germany); Marlene Kautzner, Heart Ctr. Leipzig GmbH, Univ. Leipzig (Germany); Attila Tárnok, Univ. Leipzig (Germany)

Granulocytes are the major players in innate immunity and are prognostic markers in diseases. An in-depth phenotypic characterization of granulocyte subtypes and correlation with biometry or lifestyle is so far lacking. The reason is, that either preparation of mononuclear cells was analyzed or that cells in the neutrophil window were neglected in the analysis. Here we show for the first time lymphocyte- (LL) and monocyte-like (ML) cells within the granulocyte scatter gate as new, previously unknown cell subpopulation.

Immunophenotyping of 905 healthy German adults from the LIFE study [1] was performed by 10-color flow cytometry [2]. Age of men (n=420): 56.5±14.0 years, women (n=485): 56.7±13.6 y (range of 18-81 y). Data analyzed by FlowJo v10.0.6. Values compared by Mann-Whitney-U test: men vs women, young (18-49 y) vs. elderly (50-81 y.) men, and young (19-49 y.) vs. elderly (50-81 y.) women; significance: $p<0.05$.

Within the granulocyte gate four phenotypically distinct cell types were detected (all CD45+, SSCmid-high):

LL1 CD3+,CD4+,CD8++,CD16/56+,CD38+,HLA-DR+

LL2 CD3+,CD4low,CD8+,CD38low

LL3 CD3+,CD4+,CD8-

ML1 CD3-,CD4low,CD14+,CD38+

LL2 counts were increased in men ($p=0.042$), as well as ML1 counts ($p<0.001$). Most of the cell counts were not dependent on age, except LL2 in women. In conclusion, new lymphocyte like cell types with the neutrophil scatter characteristics are reported. Counts correlate with age and gender. We plan to sort these new subtypes for further functional characterization and aim to establish them as cellular biomarkers for the early detection of various diseases.

[1] BMC Public Health. 2015;15:691; [2] Cytometry A. 2014;85(9):781

10068-4, Session 2

Stem cells as anticancer drug carrier to reduce the chemotherapy side effect

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Chemotherapy used in cancer treatments due to the lack of specificity of drugs, is associated to various and damaging side effect that have a severe impact on patient quality of life. Over the past 30 years, increasing efforts have been placed on optimizing chemotherapy dosing with the main goals of increasing antitumor efficacy while reducing drug-associated toxicity.

A novel research shows stem cells may act as a reservoir for the anticancer drug that, subsequently, may release some of its metabolites or even the drug in its original form in vicinity of cancer cells. These cells may play a dual role in controlling drug toxicity, depending on their capacity to uptake and release chemotherapeutic drugs.

In our study we showed the Dental Pulp Stem Cells DPSC, were able to

rapidly uptake Paclitaxel PTX (FIG1) and could release it in the culture medium in a time-dependent manner. Then this conditioned culture medium is transferred to the breast cancer cells MCF7. Applying Confocal Raman Microscopy, anticancer drug uptake by MCF7 is imaged and apoptosis was observed.1,2 Surprisingly MCF7 -without any direct contact with PTX- showed drug uptake. It proves the stem cells carry and deliver anticancer drug without its modification. It could be a revolution in chemotherapy to avoid the side effects and increase the drug efficacy.

10068-5, Session 2

Predicting patient response to therapy in pancreatic and breast cancer using optical metabolic imaging

Joe T. Sharick, Vanderbilt Univ. (United States); Alexandra J. Walsh, National Research Council (United States); Melinda E. Sanders M.D., Vanderbilt Univ. (United States); Mark C. Kelley, CHI Memorial Medical Group (United States); Ingrid M. Meszoely M.D., Mary A. Hooks, Alexander A. Parikh, Vanderbilt Univ. (United States); Melissa C. Skala, Morgridge Institute for Research (United States) and Univ. of Wisconsin-Madison (United States)

There is a critical need to predict effective treatments for individual cancer patients. The goal of this work is to validate Optical Metabolic Imaging (OMI) of tumor-derived organoids as a predictive drug screening platform in breast and pancreatic cancer, by relating multiphoton fluorescence lifetime imaging (FLIM) data from these organoids to clinical patient outcomes. Three-dimensional organoids were generated from core needle biopsies of breast tumors and surgically resected pancreatic ductal adenocarcinomas (PDAC). These organoids were treated with the patient's prescribed therapy, and early metabolic changes were measured using multiphoton FLIM of the metabolic co-enzymes NAD(P)H and FAD at the single-cell level. Changes were quantified using the OMI Index, a linear combination of the optical redox ratio (ratio of the fluorescence intensities of NAD(P)H to FAD), and the mean NAD(P)H and FAD fluorescence lifetimes. Organoids grew from a variety of untreated breast tumor subtypes including triple negative, HER2+, and ER+/PR+/HER2-, and early metabolic changes could be resolved at the single-cell level after only 24 hours of treatment in vitro. Surgical pathology 2-7 months after completing neoadjuvant treatment served as gold standard validation of breast cancer patient drug response. Organoids were also successfully grown from surgically resected PDAC samples, and included two subtypes of epithelial cells as well as stromal fibroblasts. Patient follow-up data after surgery and subsequent treatment was used as gold standard validation of PDAC patient drug response. This platform shows promise for predicting long-term response to therapy in breast and pancreatic cancer patients.

10068-6, Session 2

Subcellular behavior of docosanol in living cells by coherent anti-Stokes Raman scattering microscopy

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Herpes simplex virus type 1 (HSV-1) infects at least 20% of the adult population in the United States. Docosanol is an over-the-counter topical agent that inhibits HSV replication by interfering with viral entry into cells and has proved to be one of the most effective therapies for treating herpes labialis. Nevertheless, the mechanism of its action remains poorly understood and has not yet been empirically demonstrated due to the technical challenges of intracellular imaging, which limits further development of more effective treatments for HSV-1. Here we report a quantitative spatiotemporal assessment of the uptake of docosanol in living cells using coherent anti-Stokes Raman scattering (CARS) imaging. Deuterated docosanol was designed to shift the Raman signals to the a low-background region without largely altering the physical and chemical properties of the drug. Highly concentrated docosanol was found inside living cells 24 hours after drug treatment. In addition, different spatial patterns of drug accumulation were observed in different cell lines. In keratinocytes, which are the targeted cells of docosanol, the drug molecules appeared to be docking at the interior of the cell membrane, accompanied by a redistribution of lysosomes to the periphery of the cell. In contrast, the drug molecules in fibroblasts appeared to evenly distribute throughout the cytoplasm. These results suggest that this molecular imaging approach is suitable for longitudinal tracking of drug molecules in living cells and may also have implications for elucidating the mechanism by which docosanol suppresses lesion formation.

10068-7, Session 2

Functional imaging of live Zebrafish using fluorescence lifetime optical projection tomography

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Current microscopy techniques are not optimal to image fluorescence in whole live animals. We present fluorescence lifetime optical projection tomography (FLIM OPT) applied to imaging enzyme activity in live transgenic zebrafish expressing Förster Resonance Energy Transfer (FRET) biosensors.

OPT can be considered the optical equivalent to x-ray CT. Samples are rotated through 360 with images acquired at set intervals, and a back projection technique is applied to reconstruct the 3D image. It can be performed in transmission or fluorescence modes, allowing a wide range of visualisation techniques, including FLIM. Combination of OPT with FRET FLIM can therefore provide functional information in 3D. The optimal size range for OPT is mm-cm, which fills the size gap between confocal and MRI and is also the size range for zebrafish, making them an ideal model for imaging. Transgenic zebrafish expressing a Caspase 3 FRET biosensor were generated on the TraNac background (a transparent mutant) to provide live readouts of apoptosis.

We have shown that using FLIM OPT we can detect changes in Caspase 3 activity in both embryo and adult Tg(Ubi:Caspase3biosensor) zebrafish. Apoptosis was induced using 25 Gy from a 137Cs source and post irradiation an increase in fluorescence lifetime was quantified in the head region indicative of biosensor cleavage and Caspase 3 activity. Though development of compressive sensing and multiplexed imaging with two imaging arms we have applied OPT and FLIM OPT to adult zebrafish, enabling us to quickly acquire datasets so the fish can be recovered and imaged longitudinally.

10068-8, Session 2

Use of micro-optical coherence tomography to analyze barrier integrity of intestinal epithelial cells

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Harvard Medical School (United States) and Wellman Ctr. for Photomedicine (United States); Kengyeh Chu, Duke Univ. (United States); Alex D. Eaton, Massachusetts General Hospital (United States) and Mucosal Immunology and Biology Research Ctr. (United States); Bryan P. Hurley, Massachusetts General Hospital, Harvard Medical School (United States) and Mucosal Immunology and Biology Research Ctr. (United States); Guillermo J. Tearney, Massachusetts General Hospital, Harvard Medical School (United States) and Wellman Ctr. for Photomedicine (United States)

The intestinal epithelial barrier provides protection from external threats that enter the digestive system and persist beyond passage through the stomach. The effects of toxic agents on the intestinal epithelial cell monolayer have not been fully characterized at a cellular level as live imaging of this dynamic interplay at sufficient resolution to interpret cellular responses presents technological challenges. Using a high-resolution native contrast modality called Micro-Optical Coherence Tomography (?OCT), we generated real-time 3D images depicting the impact of the chemical agent EDTA on polarized intestinal epithelial monolayers. Within minutes following application of EDTA, we observed a change in the uniformity of epithelial surface thickness and loss of the edge brightness associated with the apical surface. These observations were measured by generating computer algorithms which quantify imaged-based events changing over time, thus providing parallel graphed data to pair with video. The imaging platform was designed to monitor epithelial monolayers prior to and following application of chemical agents in order to provide a comprehensive account of monolayer behavior at baseline conditions and immediately following exposure. Furthermore, the platform was designed to simultaneously measure continuous trans-epithelial electric resistance (TEER) in order to define the progressive loss of barrier integrity of the cell monolayer following exposure to toxic agents and correlate these findings to image-based metrics. This technological image-based experimental platform provides a novel means to characterize mechanisms that impact the intestinal barrier and, in future efforts, can be applied to study the impact of disease relevant agents such as enteric pathogens and enterotoxins.

10068-9, Session 2

Quantitative evaluation of blood flow obstruction in microcirculation with sidestream dark-field images

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Septic shock induces organ dysfunction by microcirculatory disturbance. Observation and quantification of microcirculation are expected to be effective for the diagnosis of septic shock. Sidestream dark-filed (SDF) imaging is a suitable technique for this purpose. It can noninvasively visualize red blood cells of microcirculation especially at sublingual area. With an SDF camera, the flow of blood cells can be captured under a good imaging condition. However, low contrast image quality under poor imaging condition and subject's body movements make the velocity estimation difficult.

In this paper, we present a stable estimation method of the blood flow velocity in microcirculation in SDF images. In the method, we first introduced a robust principal component analysis as a preprocessing. This method decomposes a motion picture into a low-rank (L) component and a sparse (S) component. Among the two components, the S component image clearly expresses the blood flow. From the S component, vessel region is easily extracted. Then, from the temporal change of the intensity profile along the vessel the blood flow velocity is estimated.

The estimation method was examined in microcirculation of septic rats. Septic shock was induced in rat by the cecal ligation and puncture. The blood flow in small intestines of sham and septic shock rats were captured by our home-made SDF camera. While septic shock rat showed the reduction in blood flow as well as microthrombosis formation, the velocity of the sham was natural referring to the literature. These results suggest that the velocity estimation was reasonable.

10068-10, Session 2

Metabolic autofluorescence imaging of head and neck cancer organoids quantifies cellular heterogeneity and treatment response

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Treatment options for head and neck cancer are limited, and can cause an impaired ability to eat, talk, and breathe. Therefore, optimized and personalized therapies could reduce unnecessary toxicities from ineffective treatments. Organoids are generated from primary tumor tissue and provide a physiologically-relevant in vitro model to measure drug response. Additionally, multiphoton fluorescence lifetime imaging (FLIM) of the metabolic cofactors NAD(P)H and FAD can resolve dynamic cellular response to anti-cancer treatment.

This study applies FLIM of NAD(P)H and FAD to head and neck cancer organoids. Head and neck cancer tissue was digested and grown in culture as three-dimensional organoids. Gold standard measures of therapeutic response in vivo indicate stable disease after treatment with cetuximab (antibody therapy) or cisplatin (chemotherapy), and treatment response after combination treatment. In parallel, organoids were treated with cetuximab, cisplatin, or combination therapy for 24 hours. Treated organoids exhibit decreased NAD(P)H lifetime ($p<0.05$) and increased FAD lifetime ($p<0.05$) compared with control organoids. Additionally, analysis of cellular heterogeneity identifies distinct subpopulations of cells in response to treatment. A quantitative heterogeneity index predicts in vivo treatment response and demonstrates increased cellular heterogeneity in organoids treated with cetuximab or cisplatin compared with combination treatment. Mapping of cell subpopulations enables characterization of spatial relationships between cell subpopulations. Ultimately, an organoid model combined with metabolic fluorescence imaging could provide a high-throughput platform for drug discovery. Organoids grown from patient tissue could enable individualized treatment planning. These achievements could optimize quality of life and treatment outcomes for head and neck cancer patients.

10068-11, Session 3

Comparative investigation of stimulus-evoked rod outer segment movement and retinal electrophysiological activity

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Transient retinal phototropism (TRP) has been predominately observed in rod photoreceptors activated by oblique visible light stimulation. Dynamic confocal microscopy and optical coherence tomography (OCT) have revealed rod outer segment (OS) movements as the physical source of TRP. However, the physiological source of TRP is still not well understood. In this study, concurrent TRP and electroretinogram (ERG) measurements disclosed a remarkably earlier onset time of the rod OS movements (≤10 ms) than that (~38 ms) of the ERG a-wave. Furthermore, comparative experiments with low sodium treatment reversibly blocked the photoreceptor ERG a-wave, which is known to reflect hyperpolarization of retinal photoreceptors, but well preserved the TRP associated rod OS

movements. Our experimental result and theoretical analysis suggested that the physiological source of TRP might be attributed to early stages of phototransduction, before the hyperpolarization of retinal photoreceptors.

10068-12, Session 3

Distinguishing between whole cells and cell debris using surface plasmon coupled emission

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Distinguishing between intact cells, dead but still whole cells, and cell debris is an important but difficult task in life sciences. The most common way to identify dead cells is using a cell-impermeant DNA binding dye, such as propidium iodide. A healthy living cell has an intact cell membrane and will act as a barrier to the dye so that it cannot enter the cell. A dead cell has a compromised cell membrane, and it will allow the dye into the cell to bind to the DNA and become fluorescent. The dead cells therefore will be positive and the live cells will be negative. The dead cells later deteriorate quickly into debris. Different pieces of debris from a single cell can be incorrectly identified as separate dead cells. Although a flow cytometer can quickly perform numerous quantitative, sensitive measurements on each individual cell to determine the viability of cells within a large, heterogeneous population, it is bulky, expensive, and only large hospitals and laboratories can afford them. In this work, we show that the distance-dependent coupling of fluorophore light to surface plasmon coupled emission (SPCE) from fluorescently-labeled cells can be used to distinguish whole cells from cell debris. Once the fluorescent labels are excited by a laser, the fluorescently-labeled whole cells create two distinct intensity rings in the far-field, in contrast to fluorescently-labeled cell debris, which only creates one ring. The distinct far-field patterns can be captured by camera and used to distinguish between whole cells and cell debris.

10068-13, Session 3

Multi-spectral optical intrinsic signal imaging (MS-OISI) combined with optical coherence tomography (OCT) detecting difference of tumor and non-tumor tissue

Kai-Shih Chiu, National Chiao Tung Univ. (Taiwan)

Optical intrinsic signal imaging (OISI) and optical coherence tomography are both widely used nowadays for neuroimaging. Recently, newly OISI combined with multi-spectral light source have been developed by Columbia University. MS-OISI with its fine resolution and high speed can provide tempo-spatial blood flow, vessel diameter, oxygenation, and even neuronal func-tional two-dimensional map in the brain. Moreover, optical coherence tomography detecting back-scattering interference light makes it possible to obtain three-dimensional structure of tissue. Depending on tumor has totally different structure and vascular pattern than normal tissue. Tumor tends to unconscionably consuming nutrition from capillary and growing out of control. Hence, neuronal structure under tissue should be differed and could be distinguished by comparing surrounding vascular parameters to normal tissue. Using functional OCT obtained three-dimensional structure, moreover, integrating different layer en-face image in order to obtain a superficial en-face for locating position with two-dimensional map from MS-OISI. Using stimulus to obtain difference of functional map from both OCT and OISI between non-tumor and tumor tissue.

10068-14, Session 3

Dynamics of cultured HeLa cells response to photodynamic treatment with Radachlorin

Irina V. Semenova, Andrey V. Belashov, Ioffe Institute (Russian Federation); Nickolai V. Petrov, ITMO Univ. (Russian Federation); Oleg S. Vasyutinskii, Anna A. Zhikhoreva, Ioffe Institute (Russian Federation); Tatyana N. Belyaeva, Elena S. Kornilova, Anna V. Salova, Institute of Cytology (Russian Federation)

Photodynamic treatment is widely applied nowadays both for therapy of various diseases, cancers in particular, and for skin improvement in cosmetology. The treatment efficacy is studied intensively on targeted tissues, with much less research being aimed for analysis of the dynamics of individual cells response to treatment. We present results on monitoring of variations in morphological characteristics of cells in the course and after photodynamic treatment.

Experiments were performed on human cervix epidermoid carcinoma HeLa cell cultures preincubated with Radachlorin photosensitizer. Morphological characteristics of the cells were recorded and monitored by means of digital holographic microscopy. High-precision measurements of phase retardation gained by probe radiation in targeted cells demonstrate changes of their volume occurred in response to treatment. The analysis is performed on the dependence of phase retardation values and dynamics upon the treatment parameters.

The photosensitizer uptake by cells and optimal duration of preincubation in the photosensitizer solution was controlled by its fluorescence using confocal fluorescence microscope. Experiments performed by digital microscopy were assisted by observations of the same cells in the optical microscope.

The observed post-treatment decrease of phase retardation evidences cells flattening which is most likely due to the cell membrane damage and leakage of cell content into the extracellular medium. The treatment efficacy is found to be depending upon the incubation time or photosensitizer concentration in solution as well as on laser fluence and irradiation duration.

10068-50, Session 3

In vivo features of melanocytic lesions: multimode hyperspectral dermoscopy, reflectance confocal microscopy, and histopathologic correlates

Fartash Vasefi, Nicholas B. MacKinnon, Spectral Molecular Imaging, Inc. (United States); Manu Jain, Miguel A. Cordova, Kivanc Kose, Milind Rajadhyaksha, Allan C. Halpern, Memorial Sloan-Kettering Cancer Ctr. (United States); Daniel L. Farkas, Spectral Molecular Imaging, Inc. (United States) and Univ. of Southern California (United States)

Motivation and background: Melanoma, the fastest growing cancer worldwide, kills more than one person every hour in the United States. Determining the depth and distribution of dermal melanin and hemoglobin adds physio-morphologic information to the current diagnostic standard, cellular morphology, to further develop noninvasive methods to discriminate between melanoma and benign skin conditions.

Purpose: To compare the performance of a multimode dermoscopy system (SkinSpect), which is designed to quantify and map in three dimensions, in vivo melanin and hemoglobin in skin, and to validate this with histopathology and three dimensional reflectance confocal microscopy (RCM) imaging.

Methods: Sequentially capture SkinSpect and RCM images of suspect lesions and nearby normal skin and compare this with histopathology reports, RCM imaging allows noninvasive observation of nuclear, cellular and structural detail in 1-5 ?m-thin optical sections in skin, and detection of pigmented skin lesions with sensitivity of ~ 90-95% and specificity of ~ 70-80%. The multimode imaging dermoscope combines polarization (cross and parallel), autofluorescence and hyperspectral imaging to noninvasively map the distribution of melanin, collagen and hemoglobin oxygenation in pigmented skin lesions.

Results: We compared in vivo features of ten melanocytic lesions extracted by SkinSpect and RCM imaging, and correlated them to histopathologic results. We present results of two melanoma cases (in situ and invasive), and compare with in vivo features from eight benign lesions. Melanin distribution at different depths and hemodynamics, including abnormal vascularity, detected by both SkinSpect and RCM will be discussed.

Conclusion: Diagnostic features such as dermal melanin and hemoglobin concentration provided in SkinSpect skin analysis for melanoma and normal pigmented lesions can be compared and validated using results from RCM and histopathology.

10068-51, Session 3

Improved heuristics for early melanoma detection using multimode hyperspectral dermoscopy

Fartash Vasefi, Nicholas B. MacKinnon, Nicholas Booth, Spectral Molecular Imaging, Inc. (United States); Daniel L. Farkas, Spectral Molecular Imaging, Inc. (United States) and Univ. of Southern California (United States)

Purpose: To determine the performance of a multimode dermoscopy system (SkinSpect) designed to quantify and 3-D map in vivo melanin and hemoglobin concentrations in skin and its melanoma scoring system, and compare the results accuracy with SIAscopy, and histopathology.

Methods: A multimode imaging dermoscope is presented that combines polarization, fluorescence and hyperspectral imaging to accurately map the distribution of skin melanin, collagen and hemoglobin in pigmented lesions. We combine two depth-sensitive techniques: polarization, and hyperspectral imaging, to determine the spatial distribution of melanin and hemoglobin oxygenation in a skin lesion. By quantifying melanin absorption in pigmented areas, we can also more accurately estimate fluorescence emission distribution mainly from skin collagen.

Results and discussion: We compared in vivo features of melanocytic lesions (N = 10) extracted by non-invasive SkinSpect and SIMSYS-MoleMate SIAscope, and correlate them to pathology report. Melanin distribution at different depths as well as hemodynamics including abnormal vascularity we detected will be discussed. We will adapt SkinSpect scoring with ABCDE (asymmetry , border, color, diameter, evolution) and seven point dermatologic checklist including: (1) atypical pigment network, (2) blue-whitish veil, (3) atypical vascular pattern, (4) irregular streaks, (5) irregular pigmentation, (6) irregular dots and globules, (7) regression structures estimated by dermatologist.

Conclusion: Distinctive, diagnostic features seen by SkinSpect in melanoma vs. normal pigmented lesions will be compared by SIAscopy and results from histopathology.

10068-15, Session 4

Automated imaging of cellular spheroids with selective plane illumination microscopy on a chip

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Selective plane illumination microscopy (SPIM) is an optical sectioning technique that allows imaging of biological samples at high spatio-temporal resolution. Standard SPIM devices require dedicated set-ups, complex sample preparation and accurate system alignment, thus limiting the automation of the technique, its accessibility and throughput. We present a millimeter-scaled optofluidic device that incorporates selective plane illumination and fully automatic sample delivery and scanning. To this end an integrated cylindrical lens and a three-dimensional fluidic network were fabricated by femtosecond laser micromachining into a single glass chip. This device can upgrade any standard fluorescence microscope to a SPIM system.

We used SPIM on a CHIP to automatically scan biological samples under a conventional microscope, without the need of any motorized stage: tissue spheroids expressing fluorescent proteins were flowed in the microchannel at constant speed and their sections were acquired while passing through the light sheet. We demonstrate high-throughput imaging of the entire sample volume (with a rate of 30 samples/min), segmentation and quantification in thick (100-300 ?m diameter) cellular spheroids.

This optofluidic device gives access to SPIM analyses to non-expert end-users, opening the way to automatic and fast screening of a high number of samples at subcellular resolution.

10068-16, Session 4

Chemoresistance assessment of ovarian cancer microtumors trapped in a microfluidic chip using a spectroscopic imaging system

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In recent years, the microfluidic community has put a lot of effort in developing on-chip bioassays to measure cell response to a given set of stimuli. Our group has developed a chip to keep submicroliter spheroids or mouse xenografts in culture on-chip and perform in vitro chemoresistance assays. Current methods to analyze chemoresistance involve marking the microtumors with fluorescent live/dead cell markers and measuring their viability after drug treatment. While confocal and two-photon microscopy provides spatial information, and flow cytometry provides molecular information, they provide limited ways to assess cell population viability

using multiple fluorophores on-chip and at multiple time-points non-destructively.

We present a liquid crystal tunable filter-based spectroscopic imaging system that can record fluorescence and transmittance spectra of samples located in the large field of view (36 mm2). Two high-grade serous ovarian cancer cell lines, OV1946 (chemosensitive) and OV90 (chemoresistant), were transfected with green fluorescent protein (GFP) and red fluorescent protein (RFP), respectively, and used in different ratios to form co-culture spheroids. Their chemoresistance to carboplatin was followed by quantifying the GFP and RFP fluorescence after treatment. Each fluorescence image was normalized to the integration time and gain, and background noise, system response, and autofluorescence were removed. Spatial intensity variations were corrected. Spectral unmixing was then applied to separate each fluorescent protein's contribution. Building upon the results presented at Photonics West 2016 (96894E), multiplexed and simultaneous quantitative imaging of co-culture spheroids is demonstrated here opening the way to chemoresistance assays on-chip where multiple molecular tags can be used simultaneously.

10068-17, Session 4

Optical metabolic imaging of human colorectal adenocarcinoma derived three-dimensional in vitro organoids for predicting potential response to therapy

Haley M. James, Sandra P. Prieto, Gage J. Greening, Timothy J. Muldoon, Univ. of Arkansas (United States)

Locally advanced adenocarcinomas located in the distal rectum are commonly treated via 5-fluorouracil (5-FU)-based neoadjuvant chemoradiation therapy (CRT). The occurrence of pre-operative pathological complete response, or the absence of any histological evidence of residual cancer, is seen in 15-27% of rectal cancer cases. Response to chemotherapeutic agents varies between patients, introducing the need for a system to predict optimal drug combinations. We propose a method of utilizing optical metabolic imaging of in vitro, primary tumor-derived, three-dimensional organoid culture to create specific drug sensitivity profiles, and to rapidly assess a patient's potential response to drugs. Murine xenografts were developed in Swiss athymic nude mice, using human colorectal adenocarcinoma cell lines, implanted in the flank (RKO, ATCC). Tumors were excised upon reaching a volume of 500mm3 and processed for organoid culture. Organoids were subjected to longitudinal metabolic imaging of metabolic cofactors FAD and NADH for seven days. The resulting images were used to yield an optical redox value on a cell-by-cell basis, determined by the fluorescence intensity ratio of FAD/(FAD+NADH). This data infers proliferative index of the organoids. Beginning on day three, a control vehicle dimethyl sulfoxide, or the cytotoxic agent 5-FU, was added to the organoid growth media in wells, with metabolic imaging performed the same as previously stated. The optical redox values decreased due to the addition of 5-FU, which targets rapidly dividing cells and induces apoptosis. The changes in the optical redox histograms were correlated to markers of cell proliferation (Ki-67) and apoptosis (cleaved caspase-3).

10068-19, Session 5

Non-destructive measurement of collagen crosslinks using label-free fluorescence lifetime imaging

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Collagen crosslinking plays a key role in determining the mechanical properties of collagen rich tissues such as bone, cartilage and blood vessel. Using our optical fiber based apparatus, label-free single-point time resolved fluorescence spectroscopy (TRFS) and multispectral fluorescence lifetime imaging (FLIm) were used to non-destructively map changes in the biochemical make-up of collagen gel samples incubated in solutions designed to induce two different collagen crosslinks. Glutaraldehyde (GTA) is widely used to crosslink collagen based biomaterials, and incubation in ribose solution has been shown to promote the development of advanced glycation end-products (AGE) including the fluorescent Pentosidine crosslink. Following excitation at 355 nm, the mean fluorescence lifetime of the crosslinked gels in the 450-490 nm spectral region changed by 0.86 ± 0.31 ns and 1.45 ± 0.07 ns for the GTA and ribose incubated samples respectively. No significant change was observed in the corresponding control groups. The mechanical properties of all samples were measured post-imaging using rheology and compression testing and confirmed the enhanced stiffness of the crosslinked samples. The non-destructive nature of TRFS and FLIm allowed longitudinal imaging of the samples, in which the development of crosslinks was monitored on a timescale of minutes. These findings suggest that a fiber based FLIm system may have applications in monitoring critical parameters in the development of engineered tissues inside a bioreactor, or in vivo post-implantation.

10068-20, Session 5

Raman analysis of vascular endothelial growth factors (VEGF), epidermal growth factor (EGF), and transforming growth factor alpha (TGF-a)

Ty Olmstead, Bryan Auz, John Rodriguez, Matthew Comstock, Ocean Optics, Inc. (United States)

During wound healing there are a variety of growth factors involved in the healing process. Some of the growth factors that may be found in the wound bed include: Vascular Endothelial Growth Factors (VEGF), Epidermal Growth Factor (EGF), and Transforming Growth Factor alpha TGF-a. VEGF is type of a protein produced by cells to stimulate vasculogenisis and angiogenesis. VEGF stimulates cells for new blood vessel development in embryonic development, new blood vessels development after injury, muscles and development of collateral circulation to bypass blocked vessels. There are several variants of VEGF including VEGF-A, VEGF-B, and VEGF-C. VEGF-A, VEGF-B, and VEGF-C are associated with angiogenesis, maintaining new vascularization, and lymphangiogenisis respectively. EGF simulates cellular proliferation, differentiation and survival. When EGF binds with TGF-a, it initiate multiple cell proliferation. In this paper, 785nm laser based polarization sensitive Raman analysis is used to characterize the Raman spectrum of VEGF-A, VEGF-B, VEGF-C, EGF, and TGF-a. Raman spectroscopy provides a means to non-destructively analyze the characteristics of these proteins without the need for optical labels. This offers the possibility of rapid assessment of growth factors in vitro. In this study, the first steps toward this are taken by measuring the polarization Raman spectral response of the individual variants of VEGF, EGF and TGF-a.

10068-18, Session PMon

Quantification of patient-derived 3D cancer spheroids in high-content screening images

Mi-Sun Kang, Ewha Womans Univ. (Korea, Republic of); Seon-Min Rhee, Samsung Advanced Institute of Technology (Korea, Republic of); Myoung-Hee Kim, Ewha Womans Univ. (Korea, Republic of)

We present a cell image quantification method for image-based drug response prediction from patient-derived glioblastoma cells. Drug response of each person differs at the cellular level. Therefore, quantification of a patient-derived cell phenotype is important in drug response prediction. We performed fluorescence microscopy to understand the features of

patient-derived 3D cancer spheroids. A 3D cell culture simulates the in-vivo environment more closely than 2D adherence culture, and thus, allows more accurate cell analysis. Furthermore, it allows assessment of cellular aggregates. Cohesion is an important feature of cancer cells. In this paper, we demonstrate image-based quantification of cellular area, fluorescence intensity, and cohesion. To this end, we first performed image stitching to create an image of each well of the plate with the same environment. This image shows colonies of various sizes and shapes. To automatically detect the colonies, we used a learning based classification algorithm. The nuclear intensity and morphological characteristics were used for individual nuclei segmentation; the fluorescence intensity and morphological features of each nucleus was measured. Next, we calculated the location correlation of each cell that is appeal of the cell density in the well environment. Finally, we compared the results for drug-treated and untreated cells. This technique could potentially be applied for drug screening and quantification of the effects of the drugs.

10068-53, Session PMon

Measuring two-dimension scattering pattern of marine submicron particles

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Suspended particles in sea water, for example?phytoplankton?have major effect on water optical properties?measurements of the scattering properties of these particles is of significant importance to analysis the natural water.

Volume scattering function (VSF),is most used in expressing the scattering properties? to acquiring accurate VSF data?especially the tiny particles which have a diameter under one micron, we design a system which have simple mechanical structure with an ellipsoidal reflector and a CCD, which is able to detected the large angle two-dimension scattering patterns of suspended submicron particles fast and accurately.

Comparing with the theoretical patterns calculated by Mie theory, we can easily recognize the characteristics of submicron polystyrene spheres of 5 different sizes(diameter 57,193,362,528 and 809nm) through their unique directional and polarized scattering properties?then rebuild their VSF for further analysis. Such laboratorial result show the next step of the research?which is using the system to investigate the natural water in the Bohai Sea?is possible and will deepen our understanding of the roles of submicron particles in sea water .

10068-54, Session PMon

Prostate cancer detection via novel label-free imaging system based on a photonic-crystal biosensor

Frank DeLuna, Consultant (United States); Mahsa Lotfollahi, The Univ. of Texas at San Antonio (United States); Xia Lu, LuZhe Sun, The Univ. of Texas Health Science Ctr. at San Antonio (United States); Jing Yong Ye, The Univ. of Texas at San Antonio (United States)

Biomarker screening for prostate-specific antigen (PSA) is the current clinical standard for detection of prostate cancer. However this method has shown many limitations, mainly in its specificity, which can lead to a high false positive rate. Thus, there is a growing need in developing a more specific detection system for prostate cancer. Using a photonic-crystal-based biosensor in a total-internal reflection configuration (PC-TIR), we demonstrate the use of refractive index (RI) to accomplish label-free detection of prostate cancer cells against non-cancerous prostate epithelial cells. The PC-TIR biosensor possesses an open microcavity, which in contrast to traditional closed microcavities, allows for easier access of analyte molecules or cells to interact with its sensing surface. In this study, an imaging system was designed using the PC-TIR biosensor to quantify cell RI as the contrast parameter for prostate cancer detection. Non-cancerous BPH-1 prostate epithelial cells and prostate cancer PC-3 cells were placed on a single biosensor and measured concurrently. Recorded image data was then analyzed through a home-built MATLAB program. Results demonstrate that RI is a suitable variable for differentiation between prostate cancer cells and non-cancerous prostate epithelial cells. Our study shows clinical potential in utilizing RI test for the detection of prostate cancer.

10068-55, Session PMon

Spheroid imaging of phase-diversity homodyne OCT

Naoko Senda, Kentaro Osawa, Hitachi, Ltd. (Japan)

Non-invasive 3D imaging technique is essential for regenerative tissues evaluation. Optical coherence tomography (OCT) is one of 3D imaging tools with no staining and is used extensively for fundus examination. We have developed Phase-Diversity Homodyne OCT which enables cell imaging because of high resolution (axial resolution; ~2.6 ?m, lateral resolution; ~1 ?m, in the air), whereas conventional OCT was not used for cell imaging because of low resolution (10-20 ?m).

We demonstrated non-invasive imaging inside living spheroids with Phase-Diversity Homodyne OCT. Spheroids are spheroidal cell aggregates and used as regenerative tissues. Cartilage cells were cultured in low-adhesion 96-well plates and spheroids were manufactured. Cell membrane and cytoplasm of spheroid were imaged with OCT. The OCT images were verified by RCM (Reflection Confocal Microscopy) images and histological staining.

10068-56, Session PMon

Optimizing a time-resolved spectrometer for all time scales

Rainer Erdmann, Manoel Veiga, Hans-Jürgen Rahn, Tino Roehlicke, Marcus Sackrow, Alexander Glatz, Christian Litwinski, Sebastian Tannert, Uwe Ortmann, PicoQuant GmbH (Germany)

Time-resolved fluorescence spectroscopy is a spectroscopist's most valuable tool for the investigation of excited state dynamics in molecules, complexes, or semi-conductors. In recent years, the study of luminescence properties has gained in popularity in many scientific fields, including Chemistry, Biology, Physics, as well as in Life, Material or Environmental Sciences.

The investigations to be carried out in each of these fields impose different requirements. On one side, monitoring dynamic processes in the excited state necessitates high time resolution that can be achieved by fast pulsed lasers and detectors along with appropriate time-correlated single photon counting (TCSPC) units and small monochromators. On the other hand, high spectral resolution is desirable for fluorophore characterization, requiring detectors with high quantum efficiencies, flash lamps for phosphorescence measurements and large monochromators. Up to now, spectrometers have been usually targeted towards either one of these two specifications.

Spectrometers equipped with hybrid detectors, versatile TCSPC cards with optional longer time ranges, and pulsed lasers capable of working in a burst mode can offer an combined solution, covering most of the demands of either high time or spectral resolution. We will demonstrate the performance of such a spectrometer in terms of its time resolution, the ability to measure long decays and record time-gated spectra using laser drivers with burst capabilities. This type of instrument is of great value for analytical facilities in research centers, as it offers a wide range of possible spectroscopic applications in a single, easy to use instrument.

10068-57, Session PMon

Near-infrared absorption gold nanomaterials as photoacoustic and ultrasonic contrast agents for biomedical imaging

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Many methods are being studied to increase the strength of photoacoustic signals, such as contrast agents, high quality transducers, advanced signal processing, etc. Photoacoustic (PA) and contrast enhanced ultrasound (CE-US) imaging have been used for tracking abnormal tumour microenvironment characteristics in vivo.

In this work, we performed an enhanced method to improve the resolution, contrast, and depth of detection by Near-Infrared Absorption gold Nanomaterials as Photoacoustic and Ultrasonic Contrast Agents.

We performed a comparative study of Photoacoustic (PA) and contrast enhanced ultrasound (CE-US) imaging as unmodified melanoma agents, gold nanoparticle (AuNP) tagged melanoma agents. Each sample was irradiated at 750 nm and 1064 nm (9mm beam diameter, 10ns pulse width, 100Hz pulse repetition rate, 20mJ/cm2 energy fluence per pulse). The transducer used to record the PA and (CE-US) signals consisted of three elements arranged in an annular array. Each melanoma sample was loaded into cylindrical tubes and placed at the focus of the ultrasonic transducer. the PA and (CE-US) signal strength (PSS) was computed as the integration of the Hilbert Transform of the PA and (CE-US) signal. The function generation and signal acquisition were performed in the PC using Labview software.

The comparison revealed that the tagged gold nanoparticles could show their viability for pre-cancerous and malignant melanoma lesions at a very low concentration. The results of our study have the potential to not only better develop Photoacoustic (PA) and contrast enhanced ultrasound (CE-US) detection of melanoma, but also extend the viability and use of Photoacoustic (PA) and contrast enhanced ultrasound (CE-US) imaging into detection of otherwise unpigmented cancers.

10068-58, Session PMon

Spectroscopic analysis of autofluorescence distribution in digestive organ for unstained metabolism-based tumor detection

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The autofluorescence spectra of coenzymes in digestive organs are analyzed for developing tumor detection imaging technique. We focus our attention on NADH and FAD, the coenzymes whose densities change depending on the oxygen partial pressure in organs. Since the oxygen partial pressure differs in normal and tumor areas because of the difference of the rate of cell division, determining the coenzyme densities enables to detect the tumor. For estimating the coenzyme densities in the digestive organs, we measured autofluorescence spectra by the originally designed confocal spectroscope into the depth direction step wise from the surface of the organs. The confocal autofluorescence spectroscope has three lasers with wavelength of 375, 405, and 450 nm for excitation and correspondent three autofluorescence spectra were measured at a point. The measurement spot was moved with a step of 0.01 mm into the depth direction and 20 points (0.2 mm in depth) were measured in total. The autofluorescence light was incident on a diffractive grating and the spectrum was detected by an original high sensitive CMOS image sensor. The autofluorescence spectra were analyzed after correcting the hemoglobin absorption which distorts the coenzyme autofluorescence spectra. We present analysis result of the autofluorescence spectra and tumor detection demonstration.

10068-59, Session PMon

Laser polarized Xe NMR and MRI at ultra-low magnetic fields

Shun Takeda, Hiroshi Kumagai, Kitasato Univ. (Japan)

The practice of magnetic resonance imaging (MRI) currently necessitates powerful, homogeneous, and confining magnets that produce fields between ?0.4 and 10 T. MRI magnet design can be substantially simplified if the imaging is performed in ultra-low magnetic fields approaching the Earth's magnetic field. Therefore, we have studied laser polarized Xe to obtain nuclear magnetic resonance (NMR) and MRI around at an ultra-low magnetic field of 0.5 mT, corresponding to a Larmor frequency of 1.76 kHz. We obtained laser induced enhancement of Xe NMR signals by spin-exchange optical pumping of mixtures of alkali-metal vapors and Xe gases with a laser at 794.6 nm that was circularly polarized. In the presentation, we will show you also the laser induced enhancement of Xe MRI by spin-exchange optical pumping.

10068-60, Session PMon

3D imaging of collagen remodeling in the human rectal mucosa using the second harmonic generation

Svetlana Z. Jovanic, Institute of Physics (Serbia)

Second harmonic generation (SHG) phenomenon was utilized, on a home-built nonlinear laser-scanning microscope (NLM), for 3D imaging of collagen protein distribution from the human colon tissues. Aim of the study was to visualize directly remodeling of the collagen in lamina propria of the colon mucosa. Hypothesis was that such remodeling of collagen may increase lamina propria stiffness, causes changes in signal transduction and thus leads to the progression of the malignant disease. Colon tissues samples were unfixed and label-free. Results wholly indicated described remodeling of the collagen: in healthy individuals, the collagen fibers were massive, closely packed and orderly organized around the crypts and throughout the lamina propria. In the lamina propria 10 cm away from tumor, the collagen fibers were thinner, disordered and loosely arranged with noticeable spaces between them. For more comprehensive study we have measured anisotropy parameter to describe collagen fiber alignment, by detecting SHG intensity with the polarization analyzer oriented parallel and orthogonal to the laser polarization. At this moment, 3D images reveal the profound remodeling of collagen of lamina propria 10 cm away from the malignant lesion. It has been found that the described technique could be used as a valuable indicator of colon tumor progression.

10068-61, Session PMon

Usage of CT data in biomechanical research

Roman A. Safonov, Anastasiya A. Golyadkina, Irina V. Kirillova, Leonid Yu. Kossovich, Saratov State Univ. (Russian Federation)

Atherosclerosis is one of the main causes of lethal outcome in developed counties. The effectiveness of treatment depends on early diagnostics of

atherosclerosis and basic knowledge of atherosclerotic plaque evolution stages. CT can provide researchers with information about geometric characteristics of the vessel and the plaque. Furthermore, there are empirical formulas which allows to obtain Young modulus and the breaking point of the tissue from Hounsfield scale values.

As the chemical composition of the plaque changes, the rigidity of the tissue can be used to assess the stage of atherosclerotic lesion.

Using mathematical methods, such as active contour model or region growing, a 3D computer patient-oriented solid-state model can be created. Then, mechanical problem is formulated using deformable solid mechanics theory for vessel wall and the plaque and fluid dynamics theory for inner volume of the vessel. The problem can be solved numerically using finite element method. The obtained results are used to analyse the biomechanical state of the vessel on different stages of atherosclerosis evolution.

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10068-62, Session PMon

Detection of mast cell degranulation by fluorescent molecular diffusion dynamic measurement

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AIM: To establish a rapid in vitro method for mast cell degranulation tracing by raster image correlation spectroscopy (RICS). METHODS: RBL-2H3, a basophilic granulocyte mast cell line transfected with CD63-GFP plasmid, was used for evaluating the methods,including ?-hexosaminidase (HEX) colorimetric assay,scanning electron microscopy (SEM) and RICS in the detection of mast cell degranulation. The sensibilities of these methods were compared. RESULTS: The sensitivities of ?-HEX colorimetric assay and SEM were 5 mg /L and 3. 9 ? 10 -2 mg /L, respectively. RICS detection showed obvious decrease in the diffusion coefficient at dose of 3. 9 ? 10-2 mg /L. CONCLUSION: Fluorescent molecular diffusion dynamic measurement can be used for rapid tracing of allergic substances in vitro. Accord-ing to the results, RICS can achieve nearly the same extent of sensitivity as the SEM does and is far more sensitive than ?-HEX colorimetric assay. Compared with SEM, RICS has several advantages: it is faster, simpler and cheaper; it can be used in living cells; it is more suitable for rapid in vitro allergenic compounds tracing. Therefore, RICS is applicable in clinic allergic antigen screening and may also be used in pharmaceutical quality control?

10068-63, Session PMon

Semiautomatic cell and bacteria counting

Yaqub Jonmohamadi, Bastian Braeuer, Frederique Vanholsbeeck, The Univ. of Auckland (New Zealand)

Counting techniques aim at estimating the number of objects in an image or video frame and have extensive applications for biomedical imaging (e.g., cell and bacteria counting in microscopic images), monitoring crowds in surveillance systems, and performing wildlife census in aerial photography. Yet, automated counting techniques using thresholding or the number of maxima are not robust enough to accurately quantify the objects due to crowding, overlapping, size discrepancy or existence of more than one object type. Here we introduce a semi-automated technique to estimate the number of the objects in an image using the total area associated with each object type. The user is required to provide a few samples (mouse click) of each object type in the image (e.g., red and white blood cells) and of the background then the algorithm identifies the maximum difference between the histogram of the sample objects and estimates the thresholds for segmentation to obtain an image with only the object of interest. The occurrence of each object is estimated by calculating the overall area (number of pixels) in the segmented image and dividing it by the mean area of the corresponding object. Current approach estimates the number of objects before individually detecting them, and indeed this is the only way to count objects when they are crowded, or they overlap or when the image resolution is poor. Our current algorithm, using simulated and real images of beads, bacteria and cells, achieves an accuracy of more than 90%.

10068-64, Session PMon

High-dynamic-range fluorescence molecular tomography

Lian Lichao, Yong Deng, Xie Wenhao, Yang Xiaoquan, Qingming Luo, Huazhong Univ. of Science and Technology (China)

We present a high-dynamic-range?HDR? fluorescence molecular tomography ?FMT? method. HDR fluorescence projection images are constructed using the recovered CCD response curve under the multiple-exposure scheme, . Image reconstruction is implemented using iterative reweighted L1 regularization by using fewer HDR fluorescence projection images.The resultas indicate that localization and quantitative accuracyof fluorescent targets with a large concentration difference is effectively improved with HDR-FMT.

10068-65, Session PMon

Multispectral imaging based on a smartphone with an external C-MOS camera for detection of seborrheic dermatitis of a scalp

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The key thing of the manuscript is the self-diagnosis of seborrheic dermatitis of a scalp at home by using multispectral imaging based on a smartphone: Because of that, we integrated a very small CMOS camera, which is typically utilized in a smartphone, with a LED modules. After that, it is directly connected to a smartphone by a OTG cable for self-diagnosis of seborrheic dermatitis of a scalp to control the device and perform spectral imaging and analysis. In fact, it was found that it is very difficult to self-diagnose seborrheic dermatitis of a scalp using our previous version of the smartphone-based multispectral imaging system. Note that the size of the developed device is less than 77 X 33 X 16 mm. Therefore, it is very compact and appropriated as a handheld device to carry out multispectral imaging of seborrheic dermatitis of a scalp for self-diagnosis of the seborrheic dermatitis using a smartphone integrated with the device. For the spectral classification and analysis of the acquired images, a smartphone App, we developed, was utilized. 3) Finally, by using the system, we performed multispectral imaging of seborrheic dermatitis regions and normal regions of a scalp and moreover discriminated between seborrheic dermatitis regions and acne or psoriasis, which looks similar to the seborrheic dermatitis regions, in the scalp. Therefore, we demonstrate that the multispectral imaging based on a smartphone have the great potentials for self-diagnosis of seborrheic dermatitis of the scalp at home.

10068-66, Session PMon

In situ temperature control and measurement with femtosecond optical tweezers: offering biomedical application

Debabrata Goswami, Dipankar Mondal, Indian Institute of Technology Kanpur (India)

We present here the control and measurement of temperature rise using femtosecond optical tweezers at near infrared (NIR) wavelengths. We have designed our experimental technique based on our theoretical development. Simultaneous application of the high temporal sensitivity of position autocorrelation and equipartition theorem enables us to elucidate temperature control and high precision measurement around the focal volume. Experimentally we constructed the benign NIR wavelength to induce local heating at low average power by adding very low fluorescent dye molecules. Local temperature control in aqueous solution excited within optically absorbing window of low quantum yield molecules is possible due to non-radiative relaxation via thermal emission. The stochastic nature of Brownian particle has enough information of its surroundings. We have mapped the nano-dimension beam waist environment by probing the fluctuation of trapped particle. At sub-micro molar concentrations, we observed temperature-rise to ~30 K from the room temperature. The gradient of temperature-rise is as sharp as the intensity of the incident pulsed laser focused by high numerical aperture objective. Thus, pulsed laser radiation always allows to develop finer surgical techniques involving minimal thermal injuries. Our new techniques with multiphoton absorbing non-fluorescent dyes can further be used for selective phototherapeutic diagnosis and eventual elimination of cancer cells due to peak power dependent nonlinear phenomenon (NLO).

10068-67, Session PMon

Efficient femtosecond driven SOX 17 delivery into mouse embryonic stem cells: differentiation studies

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Embryonic stem cells have great promise in regenerative medicine because of their ability to self-renew and differentiate into various cell types. Efficient delivery of therapeutic genes to cells rely on the use of viral vectors showing high transfection efficiencies but still raising safety issues and non-viral vectors which show poor transfection efficiencies in stem cells. In this study we use femtosecond laser pulses to optically deliver genetic material in mouse embryonic stems cells. Femtosecond laser pulses in contrast to the conventional approaches of viral vectors and non-viral vectors minimises the risk of virus induced cancer. Our results show that femtosecond laser pulses were effective in delivering Sox 17 transcription factor which resulted in the differentiation of mouse embryonic stem cells into endoderm cells. Fluorescence analysis of the samples post irradiation was performed using a multi-detection luminescent instrument and molecular assays kits which showed, decreased expression of stage specific embryonic antigen one (SSEA-1) consistent with ongoing cellular differentiation. Using PCR analysis, stem cell differentiation was confirmed by identification of expressed Sox 17 found inside the cells alongside other genes related to endoderm production. We thus concluded that laser transfection of stem cells for the purpose of differentiation, holds potential for applications in tissue engineering as a method of generating new cell lines.

10068-68, Session PMon

Testing high-power LED based light source for hyperspectral imaging microscopy

Phiwat Klomkaew, Sam A. Mayes, Thomas C. Rich, Silas J. Leavesley, Univ. of South Alabama (United States)

Our lab has worked to develop high-speed hyperspectral imaging systems that scan the fluorescence excitation spectrum for biomedical imaging applications. Hyperspectral imaging can be used in remote sensing, medical imaging, reaction analysis, and other applications. Here, we describe the development of a hyperspectral imaging system that comprised an inverted Nikon Eclipse microscope, sCMOS camera, and a custom light source that utilized a series of high-power LEDs. LED selection was performed to achieve wavelengths of 350-590 nm. To reduce scattering, LEDs with low viewing angles were selected. LEDs were surface-mount soldered and powered by an RCD. We utilized 3D printed mounting brackets to assemble all circuit components. Spectraradiometric calibration was performed using a spectrometer (QE65000, Ocean Optics) and integrating sphere (FOIS-1, Ocean Optics). Optical output and LED driving current were measured over a range of illumination intensities. A normalization algorithm was used to calibrate and optimize the intensity of the light source. The highest illumination power was at 375 nm (3370 mW/cm^2), while the lowest illumination power was at 515, 525, and 590 nm (500 mW/cm^2). Future work will focus on using two of the same LEDs to double the power and finding more LED and/or laser diodes and chips around the range. This custom hyperspectral imaging system could be used for the detection of cancer and the identification of biomolecules.

10068-69, Session PMon

Assignment of vibrational spectral bands of kidney tissue by means of low temperature SERS spectroscopy

Martynas Velicka, Vilnius Univ. (Lithuania); Migle Radzvilaite, Vilnius Univ (Lithuania); Justinas Ceponkus, Vidita Urboniene, Milda Pucetaite, Feliksas Jankevicius, Vilnius Univ. (Lithuania); Gerald Steiner, Dresden Technical University (Germany); Valdas Sablinskas, Vilnius Univ. (Lithuania)

Vibrational spectrum of a biological tissue represents mixture of the spectra of proteins, carbohydrates, amino acids and lipids. In some cases the spectral bands are partly separated and the spectra can be used for studies of some biochemical features of the tissue. Recently we showed that both – IR and SERS spectra of extracellular fluid can be used for the discrimination of normal and cancerous tissue contacting the fluid. Unfortunately, assignment of the vibrational spectral bands is very tentative due to the high density of the bands belonging to various chemical components of the fluid. That is why any technique which allows to separate the bands is beneficial. Temperature evolution of SERS spectrum of a multicomponent sample such as biological tissue is expected to be rather different from this in case of IR absorption and Raman spectroscopy since this evolution is neither Arrhenius nor Boltzmann type. The enhancement factor of the SERS bands is very sensitive to distance between metal surface and chemical groups of the molecule under interest, and the distance can be strongly temperature dependent.

In this work temperature evolution of the SERS bands of kidney tissue extracellular fluid in the 300 - 100 K temperature range was used to improve the assignment of the SERS spectral bands of extracellular fluid taken from normal and cancerous kidney tissue.

10068-70, Session PMon

Quantitative phase imaging of platelet: assessment of cell morphology and function

Irina Vasilenko, Maimonides State Classical Academy (Russian Federation) and The M. Vladimirsky Moscow Regional Research and Clinical Institute (Russian Federation); Elizaveta Vlasova, Vladislav Metelin, Maimonides State Classical Academy (Russian Federation)

It is well known that platelets play a central role in hemostasis and thrombosis, they also mediate tumor cell growth, dissemination and angiogenesis. The purpose of the present experiment was to evaluate living platelet size, function and morphology simultaneously in unactivated and activated states using Phase-Inteference Microscope “Cytoscan” (Moscow, Russia). We enrolled 30 healthy volunteers, who had no past history of aeteriosclerosis-related disorders, such as coronary heart disease, cerebrovascular disease, hypertention, diabetes or hyperlipidemia and 25 patients with oropharynx cancer.

We observed the optic-geometrical parameters of each isolated living cell and the distribution of platelets by sizes have been analysed to detect the dynamics of cell population heterogeneity. Simultaneously we identified 4 platelet forms that have different morphological features and different parameters of size distribution.

We noticed that morphological platelet types correlate with morphometric platelet parameters. The data of polymorphisms of platelet reactivity in tumor progression can be used to improve patient outcomes in the cancer prevention and treatment. Moreover morphometric and functional platelet parameters can serve criteria of the efficiency of the radio- and chemotherapy carried out.

In conclusion the computer phase-inteference microscope provides rapid and effective analysis of living platelet morphology and function at the same time. The use of the computer phase-inteference microscope could be an easy and fast method to check the state of platelets in patients with changed platelet activation and to follow a possible pharmacological therapy to reduce this phenomenon.

10068-71, Session PMon

Cardiovascular rescue effect of stem cells evaluated by SHG-2p hybrid microscopy

Xiaoqi Yang, Clemson Univ. (United States)

Mitochondrial transfer from stem cells to cardiomyocytes might have rescue effect on damaged cardiomyocytes. Evaluating this effect requires simultaneous observation of both the process of mitochondrial transfer and functional alterations after mitochondria transfer. Each of these observation requires live cell imaging.

We used our lab-built SHG-2p hybrid microscope to study the functional improvement of cardiomyocytes after mitochondrial transfer from a stem cell through a tunneling nanotube (TnT) formed between the two cells in a biochip. The dynamic SHG signal from the sarcomeric structure in a cardiomyocyte was used to directly measure the contractility of the cell, which reflects the functional effect of mitochondrial transfer, which was visualized in real time using the 2p signal obtained from the autofluorescence of NADH in the mitochondria being transferred.

In this study, primary Day 3 neonatal rat cardiomyocytes (CMs) with hypoxia treatment were seeded in a microfluidic channel of a lab-built biochip with another microfluidic channel seeded with co-cultured mesenchymal stem cells (MSCs). The specific microfluidic-channel design allowed TnT formation between the MSCs and CMs. To use the hybrid microscopy to simultaneously visualize the sarcomeric contraction and mitochondrial transfer through TnT, in addition to study and determine the optimal excitation/emission wavelength (740 nm), we developed a rapid scanning algorithm for capturing the dynamic images of contracting sarcomeres from the SHG channel and transferring mitochondria from the 2p channel. The SHG imaging results showed significant improvement of contractility in those CMs that were obtained transferred mitochondria visualized by the 2p images.

10068-72, Session PMon

Referencing techniques for high-speed confocal fluorescence lifetime imaging microscopy (FLIM) based on analog mean-delay (AMD) method

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Analog mean-delay (AMD) method is a new powerful alternative method in determining the lifetime of a fluorescence molecule for high-speed confocal fluorescence lifetime imaging (FLIM). Even though the photon economy and the lifetime precision of the AMD method are proven to be as good as the state-of-the-art time-correlated single photon counting (TC-SPC) method, there have been some speculations and concerns about the accuracy of this method. In the AMD method, the temporal waveform of an emitted fluorescence signal is directly recorded with a slow digitizer whose bandwidth is much lower than the temporal resolution of lifetime to be measured. We have found that the drifts and the fluctuations of the absolute zero position in a measured temporal waveform are the major problems in the AMD method. We have also proposed dual channel waveform measurement scheme that may suppress these errors. It is shown that there may exist more than 2 ns drift in a measured temporal waveform during the period of the first 12 minutes after electronics components are turned on. The standard deviation of a measured lifetime after this warm-up period can be as large as 51 ps without a proposed scheme. We have shown that this error can be reduced to 9 ps with our dual-channel waveform measurement method. We realized real-time confocal AMD-FLIM system with our proposed electrical referencing technique.

10068-73, Session PMon

Rapid measurement of meat spoilage using fluorescence spectroscopy

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Food spoilage is mainly caused by microorganisms, such as bacteria. The most important process of the bacteria for food spoilage is microbial metabolism. The proposed research evaluates a new method for rapidly measuring the spoilage status in muscle food such as meat using fluorescence spectroscopy. Meat is a biological tissue, which contains intrinsic fluorophores, such as tryptophan, collagen, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) etc. As meat spoils, it undergoes various chemical changes. The levels of the native fluorophores present in a sample may change. In particular, the changes in NADH and FAD are associated with microbial metabolism. Such changes may be revealed by fluorescence spectroscopy and used to indicate the degree of meat spoilage. Therefore, such native fluorophores may be unique, reliable and non-subjective indicators for the detection of spoiled meat. In this study, we measure the autofluorescence in meat samples longitudinally over a week in an attempt to develop a method to accurately detect meat spoilage using fluorescence spectroscopy. The results show that the relative concentrations of all above fluorophores change as the tissue samples kept in room temperature (~19oC) spoil. The changes become more rapidly after about two days. For the tissue sample kept in freezer (~ -12oC), the changes are much less or even unnoticeable over a week long storage.

10068-74, Session PMon

Infrared spectroscopic imaging for in silicon brain histological recognition

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In a nutshell the mammalian brain "cytoarchitecture" assembly is astonishing. The brain involve billions of cells organized with specific functional properties into a large number of distinctive regions. In each brain region the tissue is constructed by a large number of cell types: neurons, oligodendrocytes, astrocytes, microglia, and maintained by an extensive blood vessels network. The cells are organized into localized multicellular units, in the vast cortical cell layers, and the "niches" of neural stem cells. These regions are highly interconnected via axonal fibers, and the study of brain connectivity is the goal of the field called connectomics. In general, comprehensive mapping of an entire brain has not yet been achieved, and a much remains unknown about the brain's structure. So it is evident, however, the importance of vibrational hyperspectral imaging and high-performance computing to brain mapping. Infrared (IR) hyperspectral imaging provides microscopic morphologic details and multiplexed molecular specificity, due to their spectroscopic nature. Being the raw material for the starting point in the development of a brain mapping based chemometrics and signal processing techniques. In this way, the current histological imaging methods fail to capture the structural and molecular complexity of brain tissue. The focus of our research is to develop a non-dyes/staining imaging technique using IR hyperspectral imaging combined with high-performance computing to quantify the cytoarchitectural in the brain and pathophysiological conditions studies in a near future. Differently, this study would open the door to a new cosmivision evolving as a non-dyes/staining imaging.

10068-75, Session PMon

Time-lapse microscopy of lung endothelial cells under hypoxia

Shima Mehrvar, Zahra Ghanian, Univ. of Wisconsin-Milwaukee (United States); Ganesh Kondouri, Amadou K. S. Camara, Medical College of Wisconsin (United States); Mahsa Ranji, Univ. of Wisconsin-Milwaukee (United States)

Objective: This study utilizes fluorescence microscopy to assess the effect of the oxygen tension on the production of superoxide in mitochondria of fetal pulmonary artery endothelial cells (FPAECs).

Introduction: Hypoxia is a severe oxygen stress, which mostly causes irreversible injury in lung cells. In this study, ROS production level was examined in hypoxic FPAECs treated with pentachlorophenol (PCP, uncoupler). This work was accomplished by monitoring and quantifying the changes in the level of the produced superoxide in hypoxic cells before and after PCP treatment.

Materials and methods: The dynamic of the mitochondrial superoxide production in two groups of FPAECs was measured over time using time-lapse microscopy. For the first group, cells were incubated in 3% hypoxic condition for 2 hours and then continuously were exposed to hypoxic condition for imaging as well. For the second group, cells were incubated in hypoxic condition but then imaged in normal oxygen condition. Time lapse images of the cells loaded with Mito-SOX (superoxide indicator) were acquired, and the red fluorescence intensity profile of the cells was calculated. Changes in the level of the fluorescence intensity profile while they are treated with PCP indicates the dynamics of the superoxide level.

Results: The intensity profiles of the cells in the first group showed 2.7 times higher superoxide production rate than the non-treated cells in the same group. However, the production rate for the PCP-treated cells in the second group increased by 4.4 times which shows a wider dynamic range of the superoxide production.

Conclusion: Time lapse microscopy revealed that hypoxic cells have higher basal level of the superoxide. Higher basal level leads to smaller dynamic range of the superoxide generation in hypoxic cells treated with PCP. Therefore, this result suggests that hypoxia decreased electron transport chain activity in uncoupled chain.

10068-21, Session 6

Novel system for measuring giant spectral images and its application for cancer detection

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The spectral content of a sample provides important information that can be used for analysis and diagnostics in various applications varying from art preservation through forensics to pathological analysis of a tissue section. Spectral imaging is already in use for many applications, but measuring the spectral image of very large samples is a challenge that so far was not achieved. We present a novel system and method for scanning very large spectral images of microscopy samples. The system is based on capturing the information while the sample is continuously being scanned on the fly. The spectral separation is achieved through Fourier spectroscopy by using an interferometer mounted along the optical axis (no moving parts). We will describe the system and its use for pathological samples and cancer detection.

10068-22, Session 6

3D matching techniques using OCT fingerprint point clouds

Henrique S. Gutierrez da Costa, Luciano Silva, Olga R. P. Bellon, Univ. Federal do Paraná (Brazil); Audrey K. Bowden, Stanford Univ. (United States); Raphael K. Czovny, Univ. Federal do Paraná (Brazil)

Optical Coherence Tomography permits obtaining 3D fingerprints from the epidermis and dermis. These images expose a set of features that can be used for biometric identification with improved matching accuracy. These images can be used to overcome cases of Failure to Enroll (FTE) due to poor image quality and skin alterations. Three matching techniques are evaluated: Iterative Closed Points, Surface Interpenetration Measure and KH Maps. A comparative analysis of matching accuracy and a comparison with 2D techniques is presented. A comparison with 2D matching was also performed. Cases of FTE in the presence of moisture, scratches and abrasion are evaluated.

10068-23, Session 6

Interpreting fiber structure from polarization dependent optical anisotropy

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Polarized light is commonly used to detect optical anisotropies, such as birefringence, in tissues. This optical anisotropy is often attributed to underlying structural anisotropy in the tissue due to regularly aligned collagen fibers. In these cases, the amplitude of optical anisotropy is interpreted as a relative measure of the orientation anisotropy of the fibers. However, few models allow quantitative interpretation of absolute measures of the true fiber orientation distribution.

Our model uses the Mie solution to scattering of linearly polarized light from infinite cylindrical scatterers. This is expanded to include populations of scatterers with physiologically relevant size and orientation distributions. We investigated the influences of the size parameter, relative index of refraction, and collection angle on the back-scattering signal. Additionally, we compared back-scattering from Gaussian, von Mises, and experimentally derived fiber distributions. Electrospun fiber phantoms with controlled degrees of alignment and porcine heart valve leaflet were used as experimental models.

Our results showed that for certain combinations of size parameter and relative index of refraction, the amplitude of optical anisotropy changed drastically. This suggests that interpreting relative fiber structure based on the amplitude of the optical anisotropy alone is not an accurate representation of the true structural anisotropy in certain cases. Additionally, we showed that a circular von Mises distribution reproduced experimentally derived distributions most accurately, and may be more appropriate for describing real fiber distributions than the often used Gaussian normal distribution. This work allows more accurate quantification of fiber distributions when using polarization sensitive imaging systems.

10068-24, Session 6

Brain vascular image segmentation based on fuzzy local information C-means clustering

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Light sheet fluorescence microscopy (LSFM) is a powerful fluorescence microscopy technique which can provide three-dimensional vascular images of cleared mouse brain on a cellular resolution. However, the gray level in acquired LSFM images is uneven for vessel structures due to the heterogeneous fluorophores immunolabeling. This results in the increasing computation cost and difficulties to extract line structures in brain vascular images. Here, we present a vascular segmentation method based on fuzzy local information C-means clustering to enhance vessels. The main idea of our method is to cluster the pixel values of the enhanced image to extract line structures with different size. First of all, the gradient and the eigenvalues of hessian matrix of images were used to formulate vascular similarity gradient function, and then the high gradient values of the original image were amplified. Thereafter, a new L1-norm regularization optimization problem was formulated to keep the main brain vasculature. To enhance vessels structures, split bregman iteration method was applied for solving the L1-norm regularization problem. The fuzzy local information C-means clustering (FLICM) was used to cluster the pixel values of enhanced line signals to extract vessels. To validate our method, we applied LSFM to acquire intact mouse brain vessel image stacks for cleared mouse brain via a solvent-based clearing method. The results illustrated that our approach can effectively extract line structures of blood vessels with different size in LSFM images.

10068-25, Session 6

Use of Gabor filters and deep networks in the segmentation of retinal vessel morphology

Henry Leopold, John S. Zelek, Vasudevan Lakshminarayanan, Univ. of Waterloo (Canada)

The segmentation of retinal morphology has numerous applications in assessing ophthalmologic and cardiovascular disease pathologies. The early detection of many such conditions is often the most effective method for reducing patient risk. Computer aided segmentation of the vasculature has proven to be a challenge, mainly due to inconsistencies such as noise, variations in hue and brightness that can greatly reduce the quality of fundus images. Accurate fundus and/or retinal vessel maps give rise to longitudinal studies able to utilize multimodal image registration and disease/condition status measurements, as well as applications in surgery preparation and biometrics.

This paper further investigates the use of a Convolutional Neural Network as a multi-channel classifier of retinal vessels using the Digital Retinal Images for Vessel Extraction database, a standardized set of fundus images used to gauge the effectiveness of classification algorithms. The CNN has a feed-forward architecture and varies from other published architectures in its combination of: max-pooling, zero-padding, ReLU layers, batch normalization, two dense layers and finally a Softmax activation function. Notably, the use of Adam to optimize training the CNN on retinal fundus images has not been found in prior review. This work builds on prior work of the authors, exploring the use of Gabor filters to boost the accuracy of the system above 0.9419 during post processing. The mean of a series of Gabor filters with varying frequencies and sigma values are applied to the output of the network and used to determine whether a pixel represents a vessel or non-vessel.

10068-26, Session 6

Comparing methods for analysis of biomedical hyperspectral image data

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Over the past 2 decades, hyperspectral imaging technologies have been adapted to address the need for molecule-specific identification in the biomedical imaging field. Applications have ranged from single-cell microscopy to whole-animal in vivo imaging and from basic research to clinical systems. Enabling this growth has been the availability of faster, more effective hyperspectral filtering technologies and more sensitive detectors. Hence, the potential for growth of biomedical hyperspectral imaging is high, and many hyperspectral imaging options are already commercially available. However, despite the growth in hyperspectral technologies for biomedical imaging, little work has been done to aid users of hyperspectral imaging instruments in selecting appropriate analysis algorithms. Here, we present an approach for comparing the effectiveness of spectral analysis algorithms by combining experimental image data with a theoretical “what if” scenario. This approach allows us to quantify several key outcomes that characterize a hyperspectral imaging study: linearity of sensitivity, positive detection cut-off slope, dynamic range, false positive events, and the receiver operator curve (ROC). We present results of using this approach for comparing the effectiveness of several common spectral analysis algorithms for detecting weak fluorescent protein emission in the midst of strong tissue autofluorescence, as well as initial data showing that this approach can be used to select and optimize hyperspectral imaging hardware configurations. Results indicate that this approach should be applicable to a very wide range of applications, allowing a quantitative

assessment of the effectiveness of the combined biology, hardware, and computational analysis for detecting a specific molecular signature.

10068-27, Session 8

Cellular autofluorescence microscopy imaging using a spectrally programmable integrating sphere light source and a low noise CMOS sensor

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Integrating spheres are well known optical components used for the calibration of devices, measurement of optical properties of materials and as diffuse light sources. In this work we demonstrate a spectrally tunable and programmable integrating sphere light source employing nine light-emitting diodes (LEDs) in the 365 to 490 nm wavelength range. In combination with light collecting optics this integrating sphere has been successfully applied to microscopy imaging of weakly auto fluorescent biological cells. This new light source is a convenient low-cost substitute for standard mercury or tungsten halogen lamps usually used for auto-fluorescence imaging of biological tissues on inverted microscope, with superior characteristics. The integrating sphere produces a diffuse wavelength-tunable light field which has a uniform special profile at the imaging objective so that all cells are illuminated uniformly It uses a baffle at the light collection end which gives us the ability to obliterate the angular distribution of light intensities of LEDs as well as its angular dependence. A highly sensitive low noise CMOS camera has been used to collect the fluoresce from biological samples. The combined imaging system has the capability of making auto fluorescence images corresponding to at least 9 excitation wavelengths and has been used successfully to acquire auto-fluorescence images of the BV2 cell line.

10068-28, Session 8

Image-guided single cell extraction from mouse calvarium bone marrow in vivo

Christa Haase, Wellman Ctr. for Photomedicine (United States); Raphael Turcotte, Harvard Medical School (United States) and Janelia Research Campus (United States); Anthony P. Raphael, Massachusetts General Hospital (United States) and Univ. of Queensland (Australia); Toshihiko Oki, Massachusetts General Hospital (United States) and Harvard Stem Cell Institute (United States); Charles P. Lin, Wellman Ctr. for Photomedicine (United States) and Harvard Stem Cell Institute (United States)

Single-cell transcriptomics is becoming increasingly important in assessing tumor cell heterogeneity and micro-environment, with the ultimate goal of understanding and improving treatment options. Spatially resolved single-cell RNA sequencing, in particular, is vital to studying heterogeneities in cell populations as a function of localization. Bone marrow (BM) provides a favorable environment for hematologic malignancies such as acute myeloid leukemia (AML) or solid tumor cells originating from metastasis. We have developed a method to extract individual cells from the BM of live mice under image guidance. Briefly, a micro-well is etched into the mouse calvarium by plasma-mediated laser ablation, leaving a thin layer of bone above the selected BM cavity. The BM is perforated by creating a small circular opening of ~30 micrometers in diameter and cells are collected within the micro-well. A micro-pipette is used to aspirate the cell of interest and place it directly into a lysis buffer that facilitates subsequent amplification and RNA sequencing. An alternative method for targeting cells at a greater distance to the bone surface is by creating a larger opening to the BM and accessing cells directly with a micro-pipette. To establish our experimental approach, we used mouse AML cells that express a FRET-based sensor for caspase activation, to ensure cell viability throughout the experimental procedure. This minimally invasive approach allows for mouse survival after cell isolation and the study of transcriptional profiles as a function of disease progression and leukemic cell localization.

10068-29, Session 8

Spectrometer design with high efficiency for SD-OCT

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The optical spectrometer is a critical submodule for spectral-domain optical coherence tomography (SD-OCT) to obtain high-quality OCT images. Various factors, including the spectrometer optical resolution, the diffraction efficiency and the chromatic aberration should be taken into consideration when designing a high-quality SD-OCT imaging spectrometer. In this work, we report an optical spectrometer design through utilizing a transmission diffraction grating and a concave mirror as focusing optics for high-speed SD-OCT imaging for the first time, to the best of our knowledge. The designed optical spectrometer consists of a collimation lens, a transmission diffraction grating, a concave mirror as focusing optics and a line-scan detector. Within the wavelength range from 740nm to 920 nm considered in the design, the utilized transmission diffraction grating has a high diffraction efficiency, which is about 20% higher than that of the best commercially available reflective grating, while the concave mirror based focusing optics eliminated the chromatic aberration as it is insensitive to the wavelength of the laser beam. Such a combination of the transmission diffraction grating and concave mirror focusing optics helps enhance the spectrometer efficiency and image quality at the same time. In addition, a line-scan detector of 2048 pixels with each pixel having the size of 14?m x 14?m was considered for the spectrometer design. We optimized and analyzed such a design using the ZEMAX optical design software. Results showed good overall performances of the spectrometer, for example, the spot diagram and the Huygens point spread function (PSF) was small enough and the Strehl ratio can up to 0.96 in 780 nm~880 nm. With the current spectrometer configuration, the calculated depth scan ranges of 2 mm with a spectrometer resolution of 0.088 nm and the axial resolution of 1.68 μm were achieved.

10068-30, Session 8

Fluorescence lifetime imaging using a single photon avalanche diode array sensor

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Single photon detectors allows us work with the weakest fluorescence signals. Single photon arrays, combined with ps-controlled gating allow us to create image maps of fluorescence lifetimes, which can be used for in-vivo discrimination of tissue activity.

Here we present fluorescence lifetime imaging using the 'SwissSPAD' sensor, a 512-by-128-pixel array of gated single photon detectors, fabricated in a standard high-voltage 0.35 ?m CMOS process. We present a protocol for spatially resolved lifetime measurements where the lifetime can be retrieved for each pixel. We demonstrate the system by imaging patterns

of Fluorescein and Rhodamine B on test slides, as well as measuring mixed samples to retrieve both components of the decay lifetime.

The single photon sensitivity of the sensor creates a valuable instrument to perform live cell or live animal (in vivo) measurements of the weak autofluorescent signals, for example distinguishing unlabelled free and bound NADH. Our ultimate goal is to create a real time fluorescence lifetime imaging system, possibly integrated into augmented reality goggles, which could allow immediate discrimination of in vivo tissues.

10068-31, Session 8

Incubator embedded cell culture imaging system (EmSight) based on Fourier ptychographic microscopy

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Multi-day tracking of cells in culture systems can provide valuable information in bioscience experiments. We report the development of a cell culture imaging system, named EmSight, which incorporates multiple compact Fourier ptychographic microscopes with a standard multiwell imaging plate. The system is housed in an incubator and presently incorporates six microscopes, imaging an ANSI standard 6-well plate at the same time. By using the same low magnification objective lenses (NA of 0.1) as the objective and the tube lens, the EmSight is configured as a 1:1 imaging system that, providing large field-of-view (FOV) imaging (5.7 mm ? 4.3 mm) onto a low-cost CMOS imaging sensor. The EmSight improves the image resolution by capturing a series of images of the sample at varying illumination angles; the instrument reconstructs a higher-resolution image by using the iterative Fourier ptychographic algorithm. In addition to providing high-resolution brightfield and phase imaging, the EmSight is also capable of fluorescence imaging at the native resolution of the objectives. We characterized the system using a phase Siemens star target, and show four-fold improved coherent resolution (synthetic NA of 0.42) and a depth of field of 0.2 mm. To conduct live, long-term dopaminergic neuron imaging, we cultured ventral midbrain from mice driving eGFP from the tyrosine hydroxylase promoter. The EmSight system tracks movements of dopaminergic neurons over a 21 day period.

10068-32, Session 8

Single-cell analysis of radiotracers' uptake by fluorescence microscopy: direct and droplet approach

Maria Elena Gallina, Tae Jin Kim, Silvan C. Türkcan, Stanford Univ. (United States); Paul Abbyad, Santa Clara Univ. (United States); Guillem Pratx, Stanford Univ. (United States)

Radionuclides are used for sensitive and specific detection of small molecules in vivo and in vitro. Recently, radioluminescence microscopy extended their use to single-cell studies. Here we propose a new single-cell radioisotopic assay that improves throughput while adding sorting capabilities. The new method uses fluorescence-based sensor for revealing single-cell interactions with radioactive molecular markers. This study focuses on comparing two different experimental approaches.

Several probes were tested and Dihydrorhodamine 123 was selected as the best compromise between sensitivity, brightness and stability. The sensor was incorporated either directly within the cell cytoplasm (direct approach), or it was co-encapsulated with radiolabeled single-cells in oil-dispersed water droplets (droplet approach). Both approaches successfully activated the fluorescence signal following cellular uptake of 18F-fluorodeoxyglucose (FDG) and external X-rays exposure. The direct approach offered single-cell resolution and longtime stability (> 20 hours), moreover it could discriminate FDG uptake at labelling concentration as low as 300 μCi/ml. In cells incubated with Dihydrorhodamine 123 after exposure to high radiation doses (8-16 Gy), the fluorescence signal was found to increase with the depletion of ROS quenchers. On the other side, the droplet approach required higher labeling concentrations (1.00 mCi/ml), and, at the current state of art, three cells per droplet are necessary to produce a fluorescent signal. This approach, however, is independent on cellular oxidative stress and, with further improvements, will be more suitable for studying heterogeneous populations. We anticipate this technology to pave the way for the analysis of single-cell interactions with radiomarkers by radiofluorogenic-activated single-cell sorting.

10068-33, Session 8

Dielectrophoretic spectroscopy using a microscopic electrode array

Syed A. M. Kirmani, Fleming D. Gudagunti, Logeeshan Velmanickam, Dharmakeerthi Nawarathna, Ivan T. Lima Jr., North Dakota State Univ. (United States)

Dielectrophoresis (DEP) is a commonly used technique in biomedical engineering to manipulate biomolecules. DEP is defined as the force acting on dielectric particles when they are exposed to non-uniform AC electric fields. DEP effect can be divided in three categories: positive, negative, and zero force DEP. The cross-over frequency is the frequency in which the DEP force is equal to zero. The cross-over frequency depends on the conductivity and the permittivity of the particles and of the suspended medium, and it also depends on the radius of the particles. The DEP cross-over frequency has been utilized in detecting/quantifying biomolecules. A manual procedure is commonly used to estimate the cross-over frequency of biomolecules. Therefore, the accuracy of this detection method is significantly limited. To address this issue, we designed and tested an automated procedure to carry out DEP spectroscopy in dielectric particles dissolved in a conductive solution. Our method efficiently measures the effect of the DEP force through a live video feed from the microscope camera and performs real-time image processing. Then, it records the change in the fluorescence emission as the system automatically scans the electric frequency of the function generator over a specified time interval. We demonstrated the effectiveness of the method by extracting the crossover frequencies and the DEP spectrum of blue fluorescent polystyrene beads with 1000 nm diameter and green fluorescent beads with 500 nm diameter using this procedure. This approach can lead to the development of detection methods with significantly higher sensitivity than existing detection methods.

10068-34, Session 8

Characterization of stem cells differentiation using quantitative phase imaging

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We describe the use of quantitative phase imaging (QPI) [1] to classify stem cells according to their differentiation stages without any labelling.

Quantitative phase imaging techniques [1-4] are used in microscopy for imaging semi-transparent samples such as cells and tissues and gives information about the optical path difference (OPD). It generates highly contrasted without any labelling, which enables measuring morphological parameters. OPD also leads to sample dry mass and dry density measurement. The strength of those techniques is their non-invasive and

fast approach.

We will show that the high contrast brought by Quadri Wave Lateral Shearing Interferometry and artifacts free imaging allows cellular segmentation from which we can deduce different morphological parameters of interest and make quantitative (i.e. dry mass and mass density) measurements.

The study is realized on iPS cell line PFX#9 stem cell colonies. The whole colony is imaged using a low magnification screening (2.5x and 5x) with laser illumination. Then non-supervised classification methods are used to identify the differentiation stages. We demonstrate that QWLSI allows assessing the homogeneity of the stem cell population and automatically identifying undifferentiated pluripotent stem cells (PSC) in a two-dimension culture from other cells under differentiation. We also show that we can distinguish between 3 types of colonies of iPS cell line PFX#9 in function of their differentiation state and highlight differentiation points using a new image representation.

This information can be useful to detect a partly differentiated colony that shall be removed prior to passage in order to maintain or expand undifferentiated cells. This can also help studying culture medium and experimental conditions effects on stem cell growth and viability.

[1] P. Bon, G. Maucort, B. Wattellier, and S. Monneret, "Quadriwave lateral shearing interferometry for quantitative phase microscopy of living cells", Opt. Express, vol. 17, pp. 13080–13094, Jul 2009.

10068-36, Session 8

Femtosecond-laser assisted cell reprogramming

Hans Georg Breunig, JenLab GmbH (Germany); Aisada Uchugonova, Ana Batista, Karsten König, Univ. des Saarlandes (Germany) and JenLab GmbH (Germany)

Femtosecond-laser pulses can assist to transfect cells by creating transient holes in the cell membranes making them permeable for foreign genetic material. In contrast to many conventional approaches which rely on viruses to deliver genes factors into the cell interior and to facilitate the integration of these factors into the host genome, femtosecond-laser assisted transfection is a completely virus free alternative reprogramming method. It therefore avoids the risk of virus-caused insertional mutations due to a random integration of genes which significantly limits any future clinical uses. We have used femtosecond-laser assisted optoporation to introduce transcription factors into human fibroblasts to generate induced pluripotent stem cells employing an automated and completely software-controlled setup. The setup for adherent cells comprised of a femtosecond oscillator, a laser-scanning microscope with beam shaping optics as well as home-made software to control the laser illumination. With a second setup, flowing cells inside a micro flow tube were transfected by optoporation. Details of the experimental setup as well as the transfection results will be reported.

10068-49, Session 8

Fiber-based hyperspectral elastic scattering imaging (FHESI) system for non-contact cancer optical biopsy: system design, characterization and validation

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We developed fiber-based hyperspectral elastic scattering imaging (FHESI) system for non-contact, rapid acquisition of polarized spectral images of tissue in the body. According to theory, developed by Gustav Mie in 1908, it is possible to estimate the size of scattering particles – in the case of biological tissue the nuclei of the top cellular layers – by shining light on the target tissue and analyzing the wavelength-dependent intensity (spectral oscillations) and polarization output from the sample under investigation. By adjusting the polarization, the tissue depth of the sampled area can be controlled.

With the exception of the specialized fiber-based imaging and the synchronization electronics, the FHESI system was built using off-the-shelf components. The 1.5 meter-long imaging probe with 2 mm outside diameter incorporates a solid-core illumination fiber that delivers light from a computer-controlled monochromator in the range of 380 nm to 690 nm in 10 nm increments. The probe then uses its two imaging fiber bundles with crossed polarizers to acquire the reflected spectral information (32 images) in a quarter of a second from tissue in an area approximately 8 mm in diameter. The system characterization is presented including spatial and spectral resolution, polarization extinction ratio, and illumination power at the specimen.

The curve generated by subtracting the data from the two different polarization images can be mathematically correlated to the size of scattering particles within the background tissue thus enabling detection of cancer cells with enlarged nuclei when contrasted to normal tissue, such as lung or GI.

We have tested an earlier version of our device on model systems and clinical (lung disease) patients, and the improved version on chicken liver and muscle (breast) as well as pig muscle and skin to quantify and discriminate the tissue types based on their scattering properties. The rate of depolarization, degree of polarization, and estimated nuclear size are compared with results from literature.

10068-37, Session 9

Visualization of oxygen transportation in microcirculation by sidestream dark-field oximetry

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The sidestream dark-field (SDF) imaging is a noninvasive optical imaging technique allowing direct visualization of red blood cells in microvessels near tissue surfaces. To advance the capability of the SDF imaging, we have developed an image-based oximetry method for SDF imaging (SDF oximetry). This method is based on the Lambert-Beer law for two-wavelength illuminations. We developed a trial SDF device with multicolor light-emitting diodes (peak wavelengths: 470 and 527 nm) to obtain two-band images of microcirculation. Moreover, we suggested a method to correct the influences from both imaging camera characteristics and illumination bandwidth by using modified extinction coefficients of hemoglobin called average extinction coefficients (AECs).

In this study, the validity of our oximetry method is investigated by a phantom approach. In the phantom experiment, a phantom composed of agar powder, fat emulsion as scatterers, and blood-filled glass tubes was made and modified absorption coefficients were calculated. This phantom mimics biological tissues with microvessels. As a result, it was found that the scattered light from the periphery of the pseudo-blood vessel affects the image-based oxygen saturation (SO2) estimation and that the use of suitable values for AECs led to more accurate SO2 estimation.

We also conducted the animal experiments using pigs and rats. We are currently developing a software for estimating intravascular SO2 near the surface of the small intestine. The results will be presented at the conference.

10068-38, Session 9

Spatial and temporal skin blood volume and saturation estimation using a multispectral snapshot imaging camera

Maria Ewerlöf, Marcus Larsson, E. Göran Salerud, Linköping Univ. (Sweden)

Hyperspectral imaging (HSI) can estimate the spatial distribution of skin blood saturation, using visible to near-infrared light. HSI oximeters often use a liquid-crystal tunable filter, an acousto-optic tunable filter or mechanically adjustable filter wheels, which has too long response/switching times to monitor tissue hemodynamics. This work aims to evaluate a multispectral snapshot imaging system to estimate skin blood volume and saturation with high temporal and spatial resolution.

We use a snapshot imager, the xiSpec camera (MQ022HG-IM-SM4X4-VIS, XIMEA®), having 16 wavelength-specific Fabry–Perot filters overlaid on the custom CMOS-chip. The spectral bands are however substantially overlapping which needs to be taken into account for an accurate analysis.

Inverse Monte Carlo simulations are performed using a two-layered skin tissue model, defined by epidermal thickness, heamoglobin concentration and saturation, melanin concentration and spectrally dependent reduced-scattering coefficient, all parameters relevant for human skin. Simulations are weighted with the detector response of the xiSpec camera. At each spatial location in the field-of-view, we compare the simulated output to the detected diffusively reflected spectra with saturation levels in the range 0-100% to find the best fit. The imager is evaluated using arterial and venous occlusion of arm and finger for spatial and temporal variations and shows reproducible and accurate oxygenation maps at 512x272 pixels. The snapshot imaging with the xiSpec camera at predefined wavelengths, paired with an inverse Monte Carlo evaluation model, permit us to use this sensor for measuring spatially and temporally varying physiological parameters, such as skin blood volume and saturation.

10068-39, Session 9

Component analysis and synthesis of dark circles under the eyes using a spectral image

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Impression of human facial skin depends on color and condition such as spots, dark circles and acnes. For example, when dark circles are appeared under the eyes, the subject looks tired and older. Therefore, woman apply cosmetic cares to remove the dark circles. It is not clear about the relationship between color distribution and various chromophores distribution in the dark circles. Therefore, various cosmetic cares are applied empirically. If we can obtain skin components under eyes, effective cosmetic care can be applied with individually customization.

In this study, therefore, we applied the separation method of skin four components to hyperspectral image of dark circle, and the separated components are modulated and synthesized. These skin four components are melanin, oxy-hemoglobin, deoxy-hemoglobin, shading components. The synthesized images are evaluated which components are contributed into the appearance of dark circles. Estimation of these four components are implemented by comparison between measure hyperspectral image and modeled hyperspectral image using absorbance functions that indicates relationship between absorbance and chromophore concentration based Monte Carlo modeling of light transport in multi layered tissues.

Furthermore, data of five wavelengths are selected from data of thirty-one wavelengths to accelerate the computation time. The evaluation is performed subjectively for synthesized skin images under the eyes. The synthesized skin images are obtained by modulating the estimated chromophore concentration and shading. The results of the evaluation shows that, the cause of dark circles for the one subject was mainly melanin pigmentation under the eyes.

10068-40, Session 9

Excitation-scanning hyperspectral imaging as a means to discriminate various tissues types

Joshua Deal, Univ. of South Alabama (United States); Peter Favreau, Morgridge Institute for Research, Univ. of Wisconsin-Madison (United States); Carmen Lopez, Malvika Lall, University of South Alabama (United States); David Weber, Thomas Rich, Silas Leavesley, Univ. of South Alabama (United States)

Little is currently known about the fluorescence excitation spectra of disparate tissues and how these spectra change with pathological state. Current imaging diagnostic techniques have limited capacity to investigate fluorescence excitation spectral characteristics. This study utilized excitation-scanning hyperspectral imaging to perform a comprehensive assessment of fluorescence spectral signatures of various tissues.

Immediately following tissue harvest, a custom inverted microscope (TE-2000, Nikon Instruments) with Xe arc lamp and thin film tunable filter array (VersaChrome, Semrock, Inc.) were used to acquire hyperspectral image data from each sample. Scans utilized excitation wavelengths from 340 nm to 550 nm in 5 nm increments. Hyperspectral images were analyzed with custom Matlab scripts including linear spectral unmixing (LSU), principal component analysis (PCA), and Gaussian mixture modeling (GMM). Spectra were examined for potential characteristic features such as consistent intensity peaks at specific wavelengths or intensity ratios among significant wavelengths. The resultant spectral features were conserved among tissues of similar molecular composition. Additionally, excitation spectra appear to be a mixture of pure endmembers with commonalities across tissues of varied molecular composition, potentially identifiable through GMM. These results suggest the presence of common autofluorescent molecules in most tissues and that excitation-scanning hyperspectral imaging may serve as an approach for characterizing tissue composition as well as pathologic state. Future work will test the feasibility of excitation-scanning hyperspectral imaging as a contrast mode for discriminating normal and pathological tissues.

10068-41, Session 9

Using wavelength-normalized optical spectroscopy to improve the accuracy of bacteria growth rate quantification

Samantha E. McBirney, Kristy Trinh, Annie Wong-Beringer, Andrea M. Armani, The Univ. of Southern California (United States)

One of the fundamental analytical measurements performed in microbiology is monitoring and characterizing cell concentration in culture media. Measurement error will give rise to reproducibility problems in a wide range of applications, from biomanufacturing to basic research. Therefore, it is critical that the generated results are consistent. Single wavelength optical density (OD) measurements have become the preferred approach. Here, we compare the conventional OD600 technique with a multi-wavelength normalized scattering optical spectroscopy method to measure the growth rates of Pseudomonas aeruginosa and Staphylococcus aureus, two of the leading nosocomial pathogens with proven abilities to develop resistance. The multi-wavelength normalization process minimizes the impact of bacteria byproducts and environmental noise on the signal, thereby accurately quantifying growth rates with high fidelity at low concentrations.

In contrast, due to poor absorbance and scattering at 600 nm, the classic OD600 measurement method is able to detect bacteria but cannot quantify the growth rate reliably. Our wavelength-normalization protocol to detect bacteria growth rates can be readily and easily adopted by research labs, given that it only requires the use of a standard spectrophotometer and implementation of straightforward data analysis. Measuring and monitoring bacteria growth rates play a critical role in a wide range of settings, spanning from therapeutic design and development to diagnostics and disease prevention. Having a full understanding of the growth cycles of bacteria known to cause severe infections and diseases will lead to a better understanding of the pathogenesis of these illnesses, leading to better treatment and, ultimately, the development of a cure.

10068-43, Session 9

Evaluation of illumination systems for wide-field hyperspectral imaging in biomedical applications

Travis W. Sawyer, Siri A. Luthman, Sarah E. Bohndiek, Univ. of Cambridge (United Kingdom)

Hyperspectral imaging (HSI) systems collect both morphological and chemical characteristics from a sample by simultaneously acquiring spatial and spectral information. HSI has potential to advance cancer diagnostics by characterizing reflectance and fluorescence properties of a tissue, as well as extracting microstructural information, all of which are altered through the development of a tumor. Illumination uniformity is a critical pre-condition for extracting quantitative data from an HSI system. Spatial, angular, or spectral non-uniformity can cause glare, specular reflection and unwanted shading, which negatively impact statistical analysis techniques used to extract abundance of different chemical species. This is further exacerbated when imaging three-dimensional structures, such as tumors, whose appearance can cast shadows and form other occlusions. Furthermore, as HSI can be used simultaneously for white light and fluorescence imaging, a flexible system, which multiplexes narrowband and broadband illumination is necessary to fully utilize the capabilities of a biomedical HSI system. To address these challenges, we modeled illumination systems frequently used in wide-field biological imaging with the software LightTools and FRED. Each system is characterized for spectral, spatial, and angular uniformity, as well as total efficiency. While all three systems provide high spatial and spectral uniformity, the highest angular uniformity is achieved using a diffuse scattering dome, yielding a contrast of 0.503 and average deviation of 0.303 with a 3.91% model error. Nonetheless, results suggest that conventional systems may not be suitable for low-light-level, where tailoring illumination to match spatial and spectral requirements may be the best approach to maximize the performance.

10068-52, Session 9

A smartphone application for psoriasis segmentation and classification

Fartash Vasefi, Nicholas B. MacKinnon, Timothy Horita, Kevin Shi, eTreat Medical Diagnostics Inc. (Canada); Tamanna Tabassum Khan Munia, Kouhyar Tavakolian, Minhal Alhashim, Reza Fazel-Rezai, Univ. of North Dakota (United States)

Psoriasis is a chronic skin disease affecting approximately 125 million people worldwide. Currently, dermatologists monitor changes of psoriasis by clinical evaluation or by measuring psoriasis severity scores over time which lead to Subjective management of this condition. The goal of this paper is to develop a reliable assessment system to quantitatively assess the changes of erythema and intensity of scaling of psoriatic lesions.

A smartphone deployable mobile application is presented that uses the smartphone camera and cloud-based image processing to analyze physiological characteristics of psoriasis lesions, identify the type and stage of the scaling and erythema. The application targets to automatically evaluate Psoriasis Area Severity Index (PASI) by measuring the severity and extent of psoriasis. The mobile application performs the following core functions: 1) it captures text information from user input to create a profile in a HIPAA compliant database. 2) It captures an image of the skin with psoriasis as well as image-related information entered by the user. 3) The application color correct the image based on environmental lighting condition using calibration process including calibration procedure by capturing Macbeth ColorChecker image. 4) The color-corrected image will be transmitted to a cloud-based engine for image processing. In cloud, first, the algorithm removes the non-skin background to ensure the psoriasis segmentation is only applied to the skin regions. Then, the psoriasis segmentation algorithm estimates the erythema and scaling boundary regions of lesion.

We analyzed 10 images of psoriasis images captured by cellphone, determined PASI score for each subject during our pilot study, and correlated it with changes in severity scores given by dermatologists. The success of this work allows smartphone application for psoriasis severity assessment in a long-term treatment.

10068-44, Session 10

Design of next-generation, high-throughput, microfluidic flow cytometer/sorters for rare cell detection and isolation

James F. Leary, Aurora Life Technologies, LLC (United States)

Most microfluidic flow cytometers are relatively slow speed, not very portable, low throughput devices. These may be sufficient for some applications, but applications requiring analysis of relatively large numbers of cells to detect rare cell subpopulations (e.g. circulating tumor cells (CTCs)) require high-throughput fluidics with either massively parallel or exponentially staging microfluidic channels. LED excitation sources and nanophotonic sensors should be inexpensive in terms of cost, power and weight requirements if the device is to be practical and portable. Total volumes of cells sufficient for statistically meaningful sampling of rare cells require some form of front-end enrichment process. Nanophotonic sensors can count individual photons but can also be noisy, but improved signal-to-noise ratios can be obtained by pulsing the LED light sources, using locked-in amplifiers, and subtracting noise sampled in between excitation pulses.

Rare cell detection requires a sophisticated combination of positive and negative selection parameters of different colors with a minimum of two positive selection parameters and a large combination of negative selection parameters which can be combined in a cocktail of a single fluorescence color. An exponentially staging microfluidics system allows cells to be re-examined until the true-positive and true negative rates are very high and the false-positive and false-negative rates are acceptably low.

Neural network architectures allow for multivariate statistical classifications can be performed in real-time permitting the isolation of rare CTCs for molecular characterization/single cell gene analysis for subsequent design of individual patient immunotherapy or for continuous tumor purging required for autologous stem cell transplantations.

10068-45, Session 10

Optimization of an enhanced ceramic micro-filter for concentrating E.coli in water

Yushan Zhang, Tianyi Guo, Chang-qing Xu, McMaster Univ. (Canada); Lingcheng Hong, Hohai Univ. (China)

Recently lower limit of detection (LOD) is necessary for rapid bacteria detection and analysis applications such as microflow cytometer and

some lab-on-a-chip devices to expand their use in clinical practices and daily life. A critical pre-conditioning step for these applications is bacterial concentration, especially for extremely low level of pathogens. Sample volume can be largely reduced with an efficient pre-concentration process. Some approaches such as hollow-fiber ultrafiltration and electrokinetic techniques have been applied to bacterial concentration.Since none of these methods provided a concentrating method with stable recovery efficiencies, bacterial concentration still remains challenging Ceramic micro-filter can be used to concentrate the bacteria while the cross flow system keeps the bacteria in suspension. In this study, an enhanced ceramic micro-filter with 0.14 um pore size was proposed and demonstrated to optimize the concentration of E.coli and the recovery efficiency was highly approved along with a high volumetric concentration ratio (>100). Known quantities (106 to 107 CFU/liter) of E.coli cells were spiked to different amounts of phosphate buffered saline (0.1 to 1 L), and then concentrated to a final retentate of 5 ml. An average recovery efficiency of 95.3% with a standard deviation of 5.6% was achieved when the volumetric concentration ratio was 10. We also compared the E.coli recovery efficiencies with variable volumetric concentration ratios. Experiment results show that the recovery efficiency decreased to 89.3% (SD =11.5%) as the volumetric concentration radio increased to 75. These results indicate that the optimized ceramic microfiltration system can successfully concentrate E.coli cells from water with a high recovery efficiency of 90.8%.

10068-46, Session 11

Ultrafast, laser-scanning time-stretch microscopy with visible light

Wenwei Yan, Jianglai Wu, Kenneth K. Y. Wong, Kevin K. Tsia, The Univ. of Hong Kong (Hong Kong, China)

Optical time-stretch microscopy is an emerging ultrafast imaging modality achieving a line-scan rate up to MHz. Conventional time-stretch microscopy primarily relies on the use of optical fibers as the temporal dispersive elements to stretch the broadband ultrashort pulses and to enable ultrafast image encoding and capture. Therefore, previous time-stretch demonstrations were limited to the near infrared (NIR) regime, where fiber transmission loss is minimum. Not only would it hinder high diffraction-limited resolution, but also the utility in biological imaging applications, most of which employ visible light. For instance, histology and immunofluorescence stains, which are highly important in cell biology and flow cytometry, are generally incompatible with NIR light sources. Moreover, light sources typically used in time-stretch imaging have been limited to femtosecond broadband lasers, which increases instrumentation cost. To address these limitations we here demonstrate time-stretch microscopy with a wavelength insensitive and low-loss pulse-stretching technique, free-space angular-chirp-enhanced delay (FACED). Specifically, FACED is composed of a misaligned retroflection mirror pair and cylindrical lens to generate enormous angle dependent delay, which provides the time-to-space mapping essential for laser-scanning time-stretch imaging.

Using FACED, we present for the first time optical time-stretch microscopy at 532 nm with a narrow band picosecond green laser (0.15nm / 10ps). We achieved an imaging line-scan rate as high as 20 MHz. High-resolution single-cell images (e.g. microphytoplanktons and human leukemic monocytes) in an ultrafast microfluidic flow (3 m/s) were captured. The equivalent imaging throughput of 10,000-100,000 cells/sec makes it powerful for high-throughput imaging flow cytometry down in single-cell precision.

10068-47, Session 11

A combination of low-resolution Raman spectroscopy (LRRS) and rapid acquisition of mean Raman spectra for the identification of cells

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It has been shown that Raman spectroscopy provides superb ability to differentiate individual cell types, and can also be used to detect circulating tumor cells (CTCs).1 CTCs have been recently identified as a main culprit for the development of cancer metastasis in cancer patients.2 It is also well known that the presence of CTCs is negatively associated with the development of metastasis and the progression of cancer. Hence, a reliable method for CTC identification will have a major impact on cancer diagnostic, monitoring of cancer progression, and cancer therapy.

There are, however, two general problems of using Raman spectroscopy for the identification of cells. On the one hand, it is not clear from which cellular location a Raman spectrum that reliably represents the given cell should be acquired. On the other hand, the Raman signal intensity is weak, so that acquisition times of several seconds are required, prohibiting a high-throughput cell sampling.

In this work we firstly show that by rapidly scanning a diffraction-limited spot over the cell and continuously acquiring a Raman spectrum it is possible to overcome the intracellular heterogeneity of a cell. And the resulting chemometric models provide a better and more robust cell classification. Secondly, we can show that the spectral resolution of a Raman spectrum is not as crucial to distinguish between different cell types. By reducing the spectral resolution 6-fold, we can achieve a signal gain 5-fold and still reliably identify single cells.

10068-48, Session 11

Physical biosimulation with microorganisms

Dan V. Nicolau, McGill Univ. (Canada)

Many mathematical problems, e.g., cryptography, network routing, require the exploration a large number of candidate solutions. Because the time required for solving these problems grows exponentially with their size, electronic computers, which operate sequentially, cannot solve them in a reasonable time; and the proposed parallel-computation approaches, e.g., DNA-, quantum computing, present fundamental and practical problems preventing their successful implementation. In contrast, biological organisms routinely process information in parallel for essential tasks, e.g., foraging, searching for space. However, aside of their sheer complexity, parallel biological processes are difficult to harness for artificial parallel computation because of a fundamental difference: organisms process analog information, e.g., concentration gradients, while computing devices process numbers. This subtle, but important difference, coupled with the opportunity to operate with large number of motile agents in parallel, opens three possible biocomputing avenues.

Biomimetic algorithms are translations of “analog” procedures used by biological agents for various tasks, e.g., space searching, chemotaxis, etc., into mathematical algorithms. This approach, conceptually similar to other biomimetics, e.g., biomimetic materials, was used to derive fungi-inspired algorithms for searching space [1] and bacterial chemotaxis-inspired algorithms for finding the edges of geometrical patterns.[2]

Biosimulation uses the procedures of large numbers of motile biological agents, directly, without any translation to formal mathematical algorithms, thus by-passing computation-proper. The agents explore complex networks

that mimic real situations, e.g., traffic. This approach focused almost entirely on traffic optimization, using an amoeboid organisms, e.g., Physarum, placed in confined geometries, with chemotactic 'cues', e.g., nutrients in node coordinates [3].

Computing with biological agents in networks consists in the use of very large number of agents exploring microfluidics networks, designed to purposefully encode hard mathematical problems.[4] For instance, we reported[5] the foundations of a parallel-computation system in which a combinatorial problem (SUBSET SUM) is encoded into a graphical, modular network embedded in a nanofabricated planar device. Exploring the network in a parallel fashion using a large number of independent agents, e.g., molecular motor-propelled cytoskeleton filaments, solves the mathematical problem. This device uses orders of magnitude less energy than conventional computers, additionally addressing issues related to parallel computing implementation.

Conference 10069: Multiphoton Microscopy in the Biomedical Sciences XVII

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10069-1, Session 1

Multiphoton microscopy and image guided light activated therapy using nanomaterials *(Keynote Presentation)*

Paras N. Prasad, Univ. at Buffalo (United States)

This talk will focus on design and applications of nanomaterials exhibiting strong multiphoton upconversion for multiphoton microscopy as well as for image-guided and light activated therapy .1-3 Such processes can occur by truly nonlinear optical interactions proceeding through virtual intermediate states or by stepwise coupled linear excitations through real intermediate states. Multiphoton processes in biocompatible multifunctional nanoparticles allow for 3D deep tissue imaging. In addition, they can produce in-situ photon conversion of deep tissue penetrating near IR light into a needed shorter wavelength light for photo-activated therapy at a targeted site, thus overcoming the limited penetration of UV or visible light into biological media.

We are using near IR emitters such as silicon quantum dots which also exhibit strong multiphoton excitation for multiphoton microscopy. Another approach involves nonlinear nanocrystals such as ZnO which can produce four wave mixing, sum frequency generation as well as second harmonic generation to convert a deep tissue penetrating Near IR light at the targeted biological site to a desired shorter wavelength light suitable for bio imaging or activation of a therapy. We have utilized this approach to activate a photosensitizer for photodynamic therapy. Yet another type of upconversion materials is rare-earth ion doped optical nanotransformers which transform a Near IR (NIR) light from an external source by sequential single photon absorption, in situ and on demand, to a needed wavelength. Applications of these nanotransformers in multiphoton photoacoustic imaging will also be presented. An exciting direction pursued by us using these multiphoton nanoparticles, is functional imaging of brain. Simultaneously, they can effect optogenetics for regioselective stimulation of neurons for providing an effective intervention/augmentation strategy to enhance the cognitive state and lead to a foundation for futuristic vision of super human capabilities.

10069-2, Session 1

Dynamic optics for multi photon microscopy *(Keynote Presentation)*

Martin J. Booth, Univ. of Oxford (United Kingdom)

Dynamically reconfigurable optical elements, such as deformable mirrors and spatial light modulators, bring extra versatility to optical systems for the control of optical fields. Multiphoton microscopy has benefitted from the use of dynamic elements, particularly through aberration or scattering compensation. However, there are other ways in which dynamic optics can enhance the capabilities of such microscopes. We present three methods that extend the spatial and temporal control of ultrashort pulses with benefit in non-linear microscopy. This includes fast volumetric microscopy of neural activity, control of pulse front distortion, and multiplexed spatio-temporal focusing.

10069-3, Session 1

Wavefront shaping enables clearer and faster two-photon fluorescence microscopy *(Keynote Presentation)*

Na Ji, Howard Hughes Medical Institute (United States)

Principles and techniques in physics and engineering have long enabled biological discovery. In particular, recent advances in optical microscopy have led to unprecedented gains in the depth, resolution, and speed at which biological specimens can be imaged. Nevertheless, challenges remain. Biological tissues distort images and scatter light, compromising resolution and limiting imaging depth. For point-scanning multiphoton microscopy, imaging speed is also limited by label brightness and the inertia of the scanning system. Here I will discuss how we employ wavefront shaping to probe cellular dynamics at high spatiotemporal resolution in living specimens. By shaping the wavefront of the excitation light in multiphoton microscopy, we have achieved diffraction-limited spatial resolution through the entire depth of primary visual cortex, and developed a video-rate (30 Hz) volumetric imaging method that can resolve single synapses in the brain. Examples will be given of how these advanced technology enabled us to gain new insights in neurobiology.

10069-4, Session 1

Molecular imaging of melanin distribution in vivo and quantitative differential diagnosis of human pigmented lesions using label-free harmonic generation biopsy *(Keynote Presentation)*

Chi-Kuang Sun, Ming-Liang Wei, Yu-Hsiang Su, National Taiwan Univ. (Taiwan); Wei-Hung Weng M.D., Harvard Medical School (United States); Yi-Hua Liao, National Taiwan Univ. Hospital (Taiwan)

Harmonic generation microscopy is a noninvasive repetitive imaging technique that provides real-time 3D microscopic images of human skin with a sub-femtoliter resolution and high penetration down to the reticular dermis. In this talk, we show that with a strong resonance effect, the third-harmonic-generation (THG) modality provides enhanced contrast on melanin and allows not only differential diagnosis of various pigmented skin lesions but also quantitative imaging for longterm tracking. This unique capability makes THG microscopy the only label-free technique capable of identifying the active melanocytes in human skin and to image their different dendriticity patterns.

In this talk, we will review our recent efforts to in vivo image melanin distribution and quantitatively diagnose pigmented skin lesions using label-free harmonic generation biopsy. This talk will first cover the spectroscopic study on the melanin enhanced THG effect in human cells and the calibration strategy inside human skin for quantitative imaging. We will then review our recent clinical trials including: differential diagnosis capability study on pigmented skin tumors; as well as quantitative virtual biopsy study on pre- and post- treatment evaluation on melasma and solar lentigo. Our study indicates the unmatched capability of harmonic generation microscopy to perform virtual biopsy for noninvasive histopathological diagnosis of various pigmented skin tumors, as well as its unsurpassed capability to noninvasively reveal the pathological origin of different hyperpigmentary diseases on human face as well as to monitor the efficacy of laser depigmentation treatments. This work is sponsored by National Health Research Institutes.

10069-5, Session 2

Multiphoton tomography of the human eye *(Invited Paper)*

Karsten König, Univ. des Saarlandes (Germany) and JenLab GmbH (Germany)

Multiphoton tomography (MPT) is a novel label-free clinical imaging method

for non-invasive and high-resolution tissue imaging. In vivo optical histology can be realized due to the nonlinear excitation of endogenous fluorophores and second harmonic generation (SHG) of collagen. Furthermore, optical metabolic imaging (OMI) is realized by two-photon fluorescence lifetime imaging (FLIM). So far, applications of the multiphoton tomographs DermaInspect and MPTflex were limited to dermatology. Novel applications include intraoperative brain tumor imaging as well as cornea imaging. The presentation highlights the latest developments in two-photon cornea imaging of donor eyes fro transplantation as well as of patients undergoing cornea surgery.

10069-6, Session 2

Structural determinations in ovarian cancer via pixel based SHG polarization analyses *(Invited Paper)*

Kirby R. Campbell, Rajeev Chaudhary, Julia Handel, Paul J. Campagnola, Univ. of Wisconsin-Madison (United States)

Remodeling of the extracellular matrix in human ovarian cancer, can be reflected in increased collagen concentration, changes in alignment and/or up-regulation of different collagen isoforms, including Col III. Using fibrillar gel models, we demonstrate that Col I and Col III can be quantitatively distinguished by 3 distinct SHG polarization specific metrics: i) determination of helical pitch angle via the single axis molecular model, ii) dipole alignment via anisotropy, and iii) chirality via SHG circular dichroism (SHG-CD). These sub-resolution differentiations are possible due to differences in the ? helix angles of the two isoforms, which co-mingle in the same fibrils. We also investigated the mechanism of the SHG-CD response and show that unlike conventional CD, it is dominated by electric dipole interactions and is consistent with the two state SHG model. We further applied these 3 polarization resolved analyses to human normal, high risk, benign tumors, and malignant human ovarian tissues. We found that these tissues could all be differentiated by these metrics, where high grade tissues had analogous ?-helical pitch angles to the in the Col I/Col III gel model. This confirms literature suggestions based on immunofluorescence and gene expression that Col III is up-regulated in high grade ovarian cancers. The different tissues also displayed differing anisotropies, indicating the fibril assemblies are distinct and likely do not result from remodeling of existing collagen but synthesis of new collagen. Importantly, these SHG polarization methods provide structural information not otherwise possible and can serve as label-free biomarkers for ovarian and other cancers.

10069-7, Session 2

Enhancing resolution and contrast in second-harmonic generation microscopy using an advanced maximum likelihood estimation restoration method

Mayandi Sivaguru, Univ. of Illinois at Urbana-Champaign (United States); Manas Ranjan Gartia, Louisiana State Univ. (United States); David S. C. Biggs, KB Imaging Solutions LLC (United States); Mohammad M. Kabir, Barghav S. Sivaguru, Vignesh A. Sivaguru, Glenn A. Fried, Gang Logan Liu, Univ. of Illinois at Urbana-Champaign (United States); Sakthivel Sadayappan, Univ. of Cincinnati College of Medicine (United States); Kimani C. Toussaint Jr., Univ. of Illinois at Urbana-Champaign (United States)

Second-harmonic generation (SHG) microscopy is a label-free imaging technique to study collagenous materials in extracellular matrix environment with high resolution and contrast. However, like many other microscopy techniques, the actual spatial resolution achievable by SHG microscopy is reduced by out-of-focus blur and optical aberrations that degrade the amplitude of the detectable higher spatial frequencies. However, in comparison with other two-photon imaging systems like two-photon fluorescence, it is difficult to apply any PSF-engineering technique to enhance the experimental spatial resolution closer to the diffraction limit Here, we present a method to improve the spatial resolution in SHG microscopy using an advanced maximum likelihood estimation (AdvMLE) algorithm to recover the otherwise degraded higher spatial frequencies in an SHG image. Through adaptation and iteration, the AdvMLE algorithm calculates an improved PSF for an SHG image and enhances the spatial resolution, as demonstrated by a reduction of the full width at half maximum (FWHM) by ~20%. Similar results are consistently observed for biological tissues with varying SHG sources, such as gold nanoparticles, collagen and myosin in tendon and heart sarcomere respectively. By obtaining an experimental transverse spatial resolution of ~400 nm, we show that the AdvMLE algorithm brings the experimental spatial resolution closer to the theoretical diffraction limit. Our approach is suitable for adaptation to micro-nano CT and MRI imaging, which has the potential to impact diagnosis and treatment of human diseases.

10069-8, Session 2

Orientation analysis of collagen fibers in healing tendon by using second-harmonic-generation microscopy

Eiji Hase, Takeo Minamikawa, Katsuya Sato, Daisuke Yonekura, The Univ. of Tokushima (Japan); Mitsuhiko Takahashi M.D., Tokushima Prefectural Central Hospital (Japan); Takeshi Yasui, The Univ. of Tokushima (Japan)

Tendon rupture is a trauma that is difficult to fully recover from. A histological staining and a tensile testing have been used to evaluate histological and mechanical healing. However, it is difficult to perform these evaluations on the same sample due to the destructiveness and the invasiveness of these approaches. To overcome this difficulty, we have combined second-harmonic-generation (SHG) microscopy with tensile testing in a rabbit model of tendon healing. SHG microscopy has high selectivity and good image contrast with respect to the structural maturity, density, and aggregates of collagen molecule without the need for histological sectioning and staining. Therefore, this combination enables us to evaluate both histological and mechanical healing of the tendon simultaneously on the single sample. Previously, we confirmed the moderate correlation of the mean SHG light intensity with Young's modulus obtained from the tensile testing. Although this correlation may imply a potential of the mean SHG light intensity as an non-destructive indicator of the mechanical healing in the recovered tendon, SHG light intensity is often influenced by the experimental conditions such as the fluctuation of the laser light intensity and the optical property of the sample.

In this study, we performed the orientation analysis of collagen fiber in healing tendon by using SHG microscopy and investigated the correlation between the orientation parameters and Young's modulus for the same sample. Since the proposed method is independent of the SHG light fluctuation, it is more reliable indicator than the mean SHG light intensity.

10069-9, Session 3

Third harmonic generation imaging of intact human cerebral organoids to assess key components of early neurogenesis in Rett Syndrome

Murat Yildirim, Danielle Feldman, Massachusetts Institute of Technology (United States); Tianyu Wang, Dimitre G. Ouzounov, Cornell Univ. (United States); Stephanie Chou, Justin Swaney, Kwanghun Chung, Massachusetts Institute of Technology (United States); Chris Xu, Cornell

Univ. (United States); Peter T. C. So, Mriganka Sur, Massachusetts Institute of Technology (United States)

Rett Syndrome (RTT) is a pervasive, X-linked neurodevelopmental disorder that predominantly affects girls. It is mostly caused by a sporadic mutation in the gene encoding methyl CpG-binding protein 2 (MeCP2).The clinical features of RTT are most commonly reported to emerge between the ages of 6-18 months and implicating RTT as a disorder of postnatal development. However, a variety of recent evidence from our lab and others demonstrates that RTT phenotypes are present at the earliest stages of brain development including neurogenesis, migration, and patterning in addition to stages of synaptic and circuit development and plasticity. We have used RTT patient-derived induced pluripotent stem cells to generate 3D human cerebral organoids that can serve as a model for human neurogenesis in vitro. We aim to expand on our existing findings in order to determine aberrancies at individual stages of neurogenesis by performing structural and immunocytochemical staining in isogenic control and MeCP2-deficient organoids. In addition, we aim to use Third Harmonic Generation (THG) microscopy as a label-free, nondestructive 3D tissue visualization method in order to gain a complete understanding of the structural complexity that underlies human neurogenesis.

As a proof of concept, we have performed THG imaging in healthy intact human cerebral organoids cleared with SWITCH. We acquired an intrinsic THG signal with the following laser configurations: 400 kHz repetition rate, 65 fs pulse width laser at 1350 nm wavelength. In these THG images, nuclei are clearly delineated and cross sections demonstrate the depth penetration capacity (> 1mm) that extends throughout the organoid. Imaging control and MeCP2-deficient human cerebral organoids in 2D sections reveals structural and protein expression-based alterations that we expect will be clearly elucidated via both THG and three-photon fluorescence microscopy.

10069-11, Session 3

Mapping the orientation of corneal sutural lamellae by means of backward-scattered SHG microscopy

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SHG microscopy was successfully used to characterize the orientation of sutural lamellae within corneal samples by means of an epi-detection scheme. In particular, the attention was focused on the organization and orientation of corneal collagen lamellae in the first 30 um of the stromal layer below Bowman’s membrane. In fact, it is thought that the irregular orientation of these specific lamellae, an in particular the existence of so-called sutural lamellae, affects the mechanical properties of the cornea and is responsible of the overall corneal stiffness. The methodology was tested by acquiring image stacks of the central portion of the cornea in ex vivo samples of healthy corneas, keratoconic corneas and keratoconic corneas treated with cross-linking. These samples represent a good benchmark for testing the methodology, considering that keratoconus is an ophthalmic disease in which the cornea loses its stiffness and acquires an abnormal conical shape because of a different organization of sutural lamellae. The samples were first imaged and characterized on the basis of forward/backward SHG ratio, finding a different ratio in keratoconus with respect to both the healthy cornea and cross-linked keratoconus. The inclination of corneal sutural lamellae was then characterized by means of a three-dimensional correlation analysis on SHG images, acquired using a backward detection geometry. Such method provided good discrimination capabilities, demonstrating that this approach can be used not only for diagnosing keratoconus in a very early stage, but also for performing treatment follow-up on cross-linked corneas.

10069-12, Session 4

In vivo multiphoton microscopy beyond 1 mm in the brain

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Two-photon fluorescence microscopy (2PM) is widely used for in vivo brain imaging. Conventional 2PM using titanium-doped sapphire oscillators is typically limited to imaging depths less than 600 ?m due to their short excitation wavelengths (700 -1,000 nm) and low pulse energy (~10 nJ). The ideal approach for deep imaging is to use both longer wavelengths to reduce the effects of scattering by heterogeneous brain tissue and higher energy pulses such that more photons reach the excitation volume at deeper tissue depths.

We perform high-resolution, non-invasive, in vivo deep-tissue imaging of the mouse neocortex using multiphoton microscopy with a high repetition rate optical parametric amplifier (OPA). The OPA outputs 400 nJ pulse energies and is tunable from 1,100 to 1,400 nm. The tunability of the OPA is an advantage over other high-pulse-energy lasers because the OPA wavelength can be matched to the peak absorption of the target fluorophore, enabling the excitation of numerous different fluorophores. We demonstrate an imaging depth of 1,200 ?m in vasculature labeled with Texas Red and 1,160 ?m in neurons labeled with tdTomato, and perform line scans as deep as 1200 ?m to measure the blood flow speed in a single capillary. We also demonstrate deep-tissue imaging using Indocyanine Green (ICG), which is FDA approved and a promising route to translate multiphoton microscopy to human applications.

10069-13, Session 4

Neuroscience imaging enabled by new highly tunable and high peak power femtosecond lasers

Tommi Hakulinen, Spectra-Physics Rankweil (Austria); Julien Klein, Spectra-Physics (United States)

Neuroscience applications benefit from recent developments in industrial femtosecond laser technology. New laser sources provide several megawatts of peak power at wavelength of 1040 nm, which enables simultaneous optogenetics photoactivation of tens or even hundreds of neurons using red shifted opsins. Another recent imaging trend is to move towards longer wavelengths, which would enable access to deeper layers of tissue due to lower scattering and lower absorption in the tissue. Femtosecond lasers pumping a non-collinear optical parametric amplifier (NOPA) enable the access to longer wavelengths with high peak powers. The high peak power of >10 MW at 1300 nm and 1700 nm allows effective 3-photon excitation of green and red shifted calcium indicators respectively and access to deeper, sub-cortex layers of the brain. Early results include in vivo detection of spontaneous activity in hippocampus within an intact mouse brain, where neurons express GCaMP6 activated in a 3-photon process at 1320 nm.

10069-14, Session 4

In vivo longitudinal visualization of bone marrow engraftment process in mouse calvarium bone marrow with two-photon microscopy

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Bone marrow transplantation became the standard choice for treatment of many leukemias, tumors and metabolic diseases. Understanding the dynamic behavior of bone marrow niches, especially in case of bone marrow transplantation is critical to improve the efficiency of the treatment. Intravital microscopy was demonstrated to be a powerful tool to study physiological structure of bone marrow niche. However, current method of intravital microscopy has difficulty in longitudinal monitoring the same bone marrow niche site due to the invasion of the prior-imaging surgery. In this study, we introduce a method to improve the bone marrow niche imaging process and enable the longitudinal imaging of murine calvarium bone marrow. Mouse model for calvarium bone marrow imaging was made by attaching cover glass window to the calvarium bone. Longitudinal imaging of whole bone marrow engraftment process was carried out to demonstrate the advantage of our mouse model. Qualitative and quantitative analysis were also executed on the image data. The result provided a dynamic and full visualization of the bone marrow engraftment process. The study was expected to provide helpful tool for bone marrow studies and useful information for bone marrow transplantation in future.

10069-15, Session 4

Design of large field-of-view two photon microscopy for imaging mouse cortex

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Spontaneous neuronal activity has been measured at cellular resolution in mice, zebrafish, and C. elegans using optical sectioning microscopy techniques, such as light sheet microscopy (LSM) and two photon microscopy (TPM). Recent improvements in these modalities and genetically encoded calcium indicators (GECI's) have enabled whole brain imaging of calcium dynamics in zebrafish and C. elegans. However, these whole brain microscopy studies have not been extended to mice due to the limited field of view (FOV) of TPM and the cumbersome geometry of LSM. Conventional TPM is restricted to diffraction limited imaging over this small FOV (around 500 x 500 microns) due to the use of high magnification objectives (e.g. 1.0 NA; 20X) and the aberrations introduced by relay optics used in scanning the beam across the sample.

To overcome these limitations, we have redesigned the entire optical path of the two photon microscope (scanning optics and objective lens) to support a field of view of Ø7 mm with relatively high spatial resolution (<10 microns). Using optical engineering software Zemax, we designed our system with commercially available optics that minimize astigmatism, field curvature, chromatic focal shift, and vignetting. Performance of the system was also tested experimentally with fluorescent beads in agarose, fixed samples, and in vivo structural imaging. Our large-FOV TPM provides a modality capable of studying distributed brain networks in mice at cellular resolution.

10069-16, Session 4

Combined multiphoton microscopy and high resolution optical coherence tomography for intravital imaging

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Multiphoton microscopy (MPM) enables intravital microscopy of living animals with subcellular resolution. Limits of MPM are the penetration depth and photodamage of the tissues. In addition getting a full picture of the tissue needs multiple staining of the different structures. To overcome these limitations, we added to a multiphoton microscope (TrimScop II, Lavision Biotec) a high resolution optical coherence tomography system (Ganymed HR, Thorlabs), which provides volumetric reflection images with 2.2 μm axial resolution. The OCT uses two superluminescent diodes with a bandwidth of 127 nm around 930 nm and a 100 kHz scan rate spectrometer. For MPM and OCT the lateral resolution is given by the NA of microscope objective and the beam diameter in the entrance pupil, which is separately adjustable for both imaging modalities. To prevent motion artifacts, image acquisition uses a line by line switching in between MPM and OCT. Each line is scanned twice to achieve co-redistricted images.

Benefits of combined imaging are finding the exact focus position and navigation in the tissues without the need of fluorescence avoiding photobleaching and photodamage. Intravital imaging with MPM and OCT is demonstrated on murine retina and small intestine.

10069-17, Session 4

High-throughput multiphoton microscopy: imaging blood flow in a mouse brain at over 360 megapixels per second

Christopher James Rowlands, Oliver T. Bruns, Moungi G. Bawendi, Peter T. C. So, Massachusetts Institute of Technology (United States)

Single-point multiphoton microscopy is inherently speed-limited by the repetition rate of the laser, if not by the brightness of the sample. Additionally, the need to collect fluorescence from each pixel sequentially, as opposed to from all pixels simultaneously, can produce difficult-to-correct motion artifacts in fast-moving samples, and synchronizing high-speed scanning mirrors can be difficult in practice, especially at the highest resolutions where mirror positions between pixels are small. While parallelization of single-point scanning in both temporal and spatial dimensions has been achieved, the speed increase is limited and motion artifacts are still present. We present an alternative high-speed temporal focusing design that maintains the axial sectioning property of multiphoton microscopy while dramatically increasing the pixel throughput to in excess of 360 megapixels per second.

Our instrument can capture a 1920?1920 frame at 100 frames per second.

By utilizing quantum dots with extremely high multiphoton cross-sections, we use this instrument to map blood-flow dynamics in the mouse brain over large volumes, in planes of approximately 4mm diameter, and with close-to diffraction-limited imaging performance. We demonstrate an achievable penetration depth of over 200μm using a Z-stack, and resolve the entire circulatory network within the field of view, from the very smallest capillaries to the large arteries with extremely high flow rates.

In this presentation we will discuss the instrument design, the particle-imaging velocimetry algorithm, and what conclusions can be drawn from such a large dataset of mouse cerebral circulatory dynamics.

10069-18, Session 4

1300nm fiber laser system for deep tissue imaging

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Nonlinear microscopy techniques have become an important tool in bio-imaging applications, offering advantages over traditional linear microscopy techniques and new possibilities for sample investigation. Here, we present an ultrashort pulse fiber laser system designed for nonlinear bio-imaging at 1300nm to provide cost-efficient access to the benefits of deeper sample penetration and reduced sample damage at longer excitation wavelength. Femtosecond pulses around 1300nm were generated by Raman induced soliton self-frequency shift in a single-mode photonic crystal fiber (PCF), which was optimized to provide high soliton energies. Tuning the pump power launched into the PCF allowed to tune the soliton wavelength from 1100nm to >1350nm while the soliton energy reached pulse energies of up to 0.7nJ at a wavelength of 1350nm, corresponding to an average power of up to 70mW.

The ultrashort pulses at 1300nm were launched into a commercial 2-photon-fluorescence (2PEF) microscope, which was originally designed for operation around 800nm. A high NA microscope objective was used to focus the 1300nm radiation into the sample. As the objective was not designed for 1300nm operation, only 15mW were available at the sample plane. We performed deep tissue 2PEF imaging in mouse brain slices using our 1300nm fiber laser system and a commercial Ti:Sa laser at 780nm. We found that imaging at 780nm was limited to a maximum depth of around 100μm due to a strong fluorescence signal background, while at 1300nm high contrast imaging was possible more than 300μm deep into the sample.

10069-19, Session 4

Engineering next generation laser sources for non-linear imaging

Darryl McCoy, Coherent Scotland Ltd. (United Kingdom); Marco Arrigoni, Coherent, Inc. (United States)

Fifteen years ago saw the first emergence of true one-box, turn-key Ti:Sapphire laser systems for multiphoton imaging techniques. As the applications space has evolved, especially for imaging of in-vivo awake behaving animal models, users place ever increasing demand for high uptime and reliability from their laser sources.

Coherent are addressing this need by embracing both newest solid state laser technologies and advanced industrial design processes.

Firstly, we demonstrate how cutting edge fibre laser technology is providing not only state of the art femtosecond performance, but also offers extremely low maintenance, alignment free reliability and low cost of ownership. It can be shown how the technology can also scale in performance to meet evolving trends in fields such as optogenetics, and imaging ever deeper into sub cortical brain tissue.

Secondly we discuss how adoption of industrial design practices, such as Highly Accelerated Life Testing and Stress Screening (HALT/HASS) is proving highly effective in delivering remarkable reliability and performance for even the most advanced femtosecond tunable laser systems and therefore into high end imaging applications.

10069-20, Session 4

Recent developments in widely tunable and high peak power ultrafast laser sources and their adoption in biological imaging

Julien Klein, Spectra-Physics (United States)

Widely tunable ultrafast lasers have enabled a large number of biological imaging techniques including point scanning multiphoton excited fluorescence (MPEF), SHG/THG and stimulated Raman imaging. Tunable ultrafast lasers offer spectral agility, covering the entire relative transparency window in live tissue (700-1300nnm) and flexibility with multi-color, synchronized outputs to support sophisticated label free techniques (e.g. stimulated Raman modalities). More recently newly available high peak power lasers based on Ytterbium technology drive advances in two-photon light-sheet, 3 photon excited fluorescence and holographic patterning for optogenetics photo-stimulation. These laser platforms offer a unique blend of compactness, ease of use and cost efficiency, and ideally complement tunable platforms typically based on Ti:Sapphire and IR optical parametric oscillators (OPO). We present various types of ultrafast laser architectures, link their optical characteristics to key bio-imaging requirements, and present relevant examples and images illustrating their impact in biological science. In particular we review the use of ultrafast lasers in optogenetics and fast in-vivo Calcium imaging deep in mouse brain.

10069-43, Session PSun

Single molecule dynamics quantitative multi-parameter analysis by PIE FastFLIM microscopy measurements and applications *(Invited Paper)*

Yuansheng Sun, Ulas Coskun, ISS, Inc. (United States); Allan Chris Ferreon, Baylor College of Medicine (United States); Beniamino Barbieri, Shih-Chu Jeff Liao, ISS, Inc. (United States)

PIE FastFLIM microscopy allows the quantitative multi-parameter measurement of single molecule protein folding and dynamics . Using donor-acceptor FRET pair-labeled proteins, we detect changes in protein conformation and dynamics by monitoring FRET efficiency, stoichiometry from analysis of donor/acceptor fluorophore intensity, and lifetime. Together with anisotropy decay information, we acquire rotational relaxation times for single molecules. By applying antibunching, FLCS and burst analysis, multi-parameters such as copy numbers in protein complexes, diffusion coefficient and molecular brightness can be fitted for deeper understanding of the conformational dynamic behavior of single protein molcules.

10069-68, Session PSun

Towards in vivo breast skin characterization using multiphoton microscopy

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Breast cancer, the second most common type of cancer in women worldwide, as well as its treatment (e.g. radiation therapy) can affect the human skin. Multiphoton microscopy (MPM) could provide new insights on the skin alterations induced by this pathology and its treatment non-invasively and with high-resolution.

The breast and forearm skin of healthy volunteers, both female and male, were imaged using the multiphoton certified clinical imaging tomograph MPTflex. Images were acquired in consecutive weeks to assess the influence of hormonal variations on the skin properties. Both breasts were considered and up to three different areas were imaged in each session.

We evaluated the feasibility of using the skin endogenous autofluorescence and second-harmonic generation to characterize the human breast skin, despite of breathing artifacts. Skin properties, such as epidermal thickness, appear to be influenced by hormonal variations in female volunteers. These alterations occur both in the breast and forearm skin.

10069-69, Session PSun

High-speed focal plane control via a liquid-crystal spatial light modulator for light sheet microscopy

Chenyang Wen, Yina Chang, Chenglin Gu, Shih-Chi Chen, The Chinese Univ. of Hong Kong (Hong Kong, China)

In this work, we present a new method to perform high-speed axial scanning for selective plane illumination microscopy (SPIM) by synchronizing the detection focal plane with excitation plane using a liquid crystal-spatial light modulator (LC-SLM). The SPIM is known as a milestone in microscopy, where samples are illuminated by a thin light sheet at the focal plane of an objective lens orthogonal to the illumination axis. Although volumetric imaging may be achieved by scanning the light sheet with a galvanometric scanner, it is often accompanied by defocus at the detection plane. To address this issue, the scanning of the light sheet and the objective lens are typically synchronized using a piezoelectric objective scanner, or alternatively, an electrical tunable lens. However, due to the limited speed (10s - 100s Hz) of these devices, it is difficult to further increase the axial scanning speed for the volumetric imaging.

In our SPIM, translation of the detection focal plane is achieved by first projecting the emissions to the back of the galvanometric mirror, which subsequently scans the emission beam across an LC-SLM, where different regions are coded with designed phase modulating patterns. Accordingly, the transverse scanning performed by the galvanometric scanner is effectively converted to axial scanning. Synchronization is automatically achieved as two sides of the galvanometric mirror are simultaneously used to scan the illumination and emission beams. Practically, axial scanning speed is equivalent to the speed of the galvanometric scanner, i.e., 10s kHz, which enables video-rate volumetric imaging for SPIM.

10069-70, Session PSun

Femtosecond two-photon absorption spectra and permanent electric dipole moment change of tryptophan, 2-aminopurine and related intrinsic and synthetic fluorophores

Aleksander K. Rebane, Alexander Mikhaylov, Jake R. Lindquist, Patrik R. Callis, Montana State Univ. (United States); Bern Kohler, Montana State Univ. (United States) and The Ohio State Univ. (United States); Juri Pahapill, Sophie de Reguardati, Matt Rammo, Merle Uudsemaa, Aleksander Trummal, National Institute of Chemical Physics and Biophysics (Estonia)

Intrinsic chromophores and their fluorescent analogs such as Tryptophan (Trp) and 2-aminopurine (2-AP) are widely used as spectroscopic probes to study the structure and function of biomolecules. Recently there has been increasing interest in utilizing these highly environment-sensitive probes for two-photon excited fluorescence (2PEF) microscopy and other nonlinear-optical imaging applications. For most of these systems, however, there is currently insufficient and in part inconsistent data about the two-photon absorption (2PA) cross section and its wavelength dependence. We report a comprehensive study of the femtosecond 2PA spectra of L-Trp, 2-AP, isoxanthopterin (IXP), 3-methylindole (3-MI) and 7-methylguanosine (m7G) in ?exc = 540 - 650 nm excitation wavelength range using both 2PEF and nonlinear transmission technique. The maximum 2PA cross section of L-Trp and 3-MI in aqueous solution at ?exc = 580nm is, correspondingly, 0.2 GM and 0.3 GM (1 GM =10?50 cm4 s photon?1), while in the other systems studied the maximum 2PA cross section was in the range 0.1 – 2.0 GM. For all systems we find that in the long-wavelength region the 2PA spectral profiles follows the one-photon absorption spectral profile. This allows using two-level model for estimation of the permanent electric dipole moment change upon the transition from ground state to the lowest-energy excited singlet electronic state. The corresponding values for L-Trp and 3-MI in aqueous solution are 1.9 -2.0 D, while for other systems the dipole change was in the range 1.0 - 3.3 D. Our findings are corroborated by quantum-chemical calculations.

10069-71, Session PSun

Analysis of interaction patterns by e-FRET images autocorrelation

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Förster resonance energy transfer measurement (FRET) is the favorite technique for the detection of molecular interactions in living cells and tissues. Fluorescence lifetime measurement in time domain (TD-FLIM) using time single photon counting (TCSPC) hardware is the most efficient method for FRET analysis in living cells. The information thus collected is fluorochrome concentration-independent with a high spatial resolution. Current techniques allow for statistical analysis of average lifetimes and FRET efficiencies (e-FRET), thus characterizing the level of interaction

between the molecules studied. However, the spatial organization of e-FRET i.e. molecular interactions, has been so far discussed on individual images without statistical approach. We have developed an autocorrelation technique for the analysis of e-FRET texture that takes advantage of the information about spatial distribution of e-FRET in a series of images. This autocorrelation technique is derived from the image correlation spectroscopy (ICS) previously developed by Wiseman et al. Lifetime images are analyzed by the MAPI software with the phasor technique (Leray et al.) and e-FRET patterns are processed by a homemade software JUPITER. From the automatic analysis of a series of images, this technique extracts two parameters characterizing the e-FRET distribution in cells: the average area and the density of e-FRET clusters. We use this technique to characterize the patterns of interactions between the Positive Transcription Elongation Factor (P-TEFb), the RNA polymerase II and Histone 2A.

10069-72, Session PSun

Simultaneous acquisition of trajectory and fluorescence lifetime of moving single particles

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Fluorescence lifetime imaging (FLIM) has been a powerful tool in life science because it can reflect all effects that involve energy transfer between an excited molecule and its environment. The combination of two-photon excitation (TPE) and time-correlated single photon counting (TCSPC) provides the ability of optical sectioning, high time resolution and detection efficiency. In some specific biological applications, it is required to record lifetime variation of moving particles, which may provide information on the change of environmental parameter along the trails of the particles.

In previous work, we have introduced a two-dimensional acousto-optic deflector (AOD) into TCSPC-based FLIM to achieve fast and flexible FLIM. In this work, we combined our FLIM system with a single-particle tracking (SPT) setup and algorithm and developed a novel particle tracking FLIM system. Using this system, we could acquire the trajectory and fluorescence lifetime of a specific moving particle simultaneously. We acquired a series of images of the target moving particle in real-time and calculated its location in each step using an SPT algorithm. The coordinates of the particle was used to control AOD scanning, which was restricted to a small area around the particle to map the fluorescence lifetime. A lifetime-marked trajectory of the moving particle was reconstructed. In the demonstration experiment, we used fluorescence beads as samples. The results indicated the potential of this technique for studying the interaction between specific moving biological macromolecules and the surrounding environment in living cells.

10069-73, Session PSun

Compressed sensing aided wide-field multiphoton microscopy

Jong Kang Park, Dushan N. Wadduwage, Christopher J. Rowlands, Peter T. C. So, Massachusetts Institute of Technology (United States)

Multiphoton excited fluorescence microscopy has become a ubiquitous tool in biological imaging mainly due to its intrinsic optical sectioning capability and reduced photo-damage, but the limited imaging speed imposed by point-by-point scanning using a galvo mirror impedes the investigation of fast biological dynamics such as neuronal activity. Parallelization of the excitation process is a promising approach to increase the imaging speed. Temporal focusing is a method for achieving this parallelization; it works by dispersing a femto-second laser pulse with a grating, subsequently achieving simultaneous spatio-temporal overlap of the spectral components at an image plane. However, the imaging depth of temporal focusing is low compared to point scanning because scattering of the emission photons means that the resulting image is rapidly blurred as the tissue penetration increases. To overcome this limitation, we have designed a compressed sensing aided temporal focusing approach to further increase the limit of temporal focusing imaging. We have developed a system in which 2D patterns (such as Hadamard patterns, which are widely used in compressed sensing) are projected onto the image plane. By projecting multiple patterns and recording the resulting fluorescence intensity on the camera, the distribution of fluorophores may be recovered. In addition to this increase in penetration depth, imaging speed may also be increased by performing compressed sensing, with the random patterns as the 2D basis set, and reconstructing using l1 minimization.

10069-74, Session PSun

Optimizing ultrafast wide field-of-view illumination for high-throughput multi-photon imaging and screening of mutant fluorescent proteins

Aleksander K. Rebane, Caleb Stoltzfus, Montana State Univ. (United States)

Two- and three-photon absorption (2PA, 3PA) induced fluorescence is an important tool for deep-tissue microscopic imaging of the brain, especially in conjunction with genetically-encoded probes such as fluorescent proteins (FPs). Unfortunately, the multi-photon absorption efficiency of available FPs is notoriously low, and augmenting the related photo physical properties e.g. by directed evolution approaches has been hindered due to lack of high-throughput screening required for the selection of multi-photon-enhanced variants.

It is well known that the rate of one-photon excited fluorescence emission increases in proportion to the average incident excitation photon flux, such that a simple lamp or LED can be conveniently used for wide field-of-view (FOV) and high-throughput fluorescence imaging and screening of mutant FPs growing on a Petri dish. In contrast, to carry out a similar screening procedure with multi-photon excitation requires high peak photon flux (>1028 photons/ cm2 s), which can be achieved only with ultrafast lasers. Even so, because the illuminating laser beam needs to be distributed over a large sample area, the amount fluorescence signal reaching the detector such as a CDD is generally prohibitively low. We report on the construction, testing and detailed photon budget analysis of a wide FOV multi-photon fluorescence imaging system. We show that to achieve minimum practical screening throughput of 1 cm2/s, one needs to optimize simultaneously the femtosecond laser power, the laser pulse repetition rate as well as the beam focusing and beam scanning procedure. We present a wide FOV 2PA-excited fluorescence imaging system using 800 nm 1-kHz regenerative-amplified Ti:sapphire femtosecond laser as well as fs optical parametric amplifier tunable over broad range of near-IR excitation wavelengths that we apply to high-throughput screening of mutant EGFPs.

10069-75, Session PSun

Correlative label-free two-photon fluorescence microscopy and polarized light imaging for 3D reconstruction of myelinated fibers orientation

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Jülich GmbH (Germany) and C. and O. Vogt Institut für Hirnforschung, Heinrich-Heine-Univ. Düsseldorf (Germany); Francesco S. Pavone, Lab. Europeo di Spettroscopie Non-Lineari, Univ. degli Studi di Firenze (Italy) and Istituto Nazionale di Ottica, Consiglio Nazionale delle Ricerche (Italy)

Connectomics aims at identifying the interactions of brain regions through the reconstruction of the neuronal fiber network. 3D-Polarized light imaging (3D-PLI) allows to assess fiber orientation and inclination angles in histological brain sections based on measurements of the birefringent properties of the myelin sheaths. This optical method enables a fast analysis of whole human brains without any exogenous labeling. A single 3D (fiber) orientation vector is obtained for each voxel and reflects the net effect of all comprised fibers. In this work, we employ an integrated dual approach that combines 3D-PLI with two-photon fluorescence microscopy (TPFM) to study the mixture of various fiber orientations within the sample (and voxel) of interest. We exploit the higher axial and radial resolution of TPFM optical sectioning in combination with myelin autofluorescence to perform the 3D reconstruction of fiber orientation within each brain section. The correlation of data obtained by the two methods permits to reconstruct areas of the brain at high resolution, below the one-micrometer level. Such approach provides a novel tool for 3D reconstruction of nerve fiber orientations in postmortem tissue. The integration of different techniques opens new perspectives of an in-depth analysis of brain connectomics, linking the submillimeter-organisation of fibers to large tracts.

10069-76, Session PSun

Evaluating annular beams in multiphoton microscopy using tissue phantoms and excised human skin

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The maximum imaging depth in conventional multiphoton microscopy is ultimately limited by noise from out-of-focus fluorescence. When imaging deep in turbid media, laser power has to be increased to a level which breaks the inherent confocality of the technique, as out-of-focus fluorescence is generated by multiphoton processes at the sample surface and at shallow depths. This undesired signal lowers the image contrast and eventually overwhelms the signal from the focal plane. In this paper, annular laser beams are explored as a concept to reduce this background signal. The approach has been theoretically verified by computer simulations, and proof-of-principle demonstrated experimentally by adopting wavefront control using a spatial light modulator, and implemented for imaging tissue phantoms simulating turbid media. Results from computational models have demonstrated a ratio of ring size to objective back aperture size of 0.4 provides an ideal balance between maintaining high axial resolution and reducing out of focus fluorescence. In the material presented here, the experiments are further expanded to include data from excised human skin ex vivo. The signal-to-background ratios were calculated and compared to images acquired with a traditional, filled-aperture Gaussian beam. Experiments in tissue phantoms show an improvement in imaging contrast of up to 30% when using annular beam illumination in comparison to Gaussian illumination. The experiments in excised tissue indicate that a similar improvement is possible in biological samples.

10069-77, Session PSun

All fiber nonlinear microscopy at 1550 nm using a double-clad fiber coupler

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Nonlinear microscopy has already shown its impact in biological research, namely in the fields of neurobiology, immunology, cancer research and embryology. Typically, these microscopes operate under free space propagation, using a dichroic mirror to separate the nonlinear signals from the excitation laser. We propose a robust all-fiber nonlinear microscopy system at 1550nm using a femtosecond fiber laser. As the sectioning is performed through nonlinear effects, nonlinear microscopy does not require a detection pinhole. Therefore, an all fiber system could benefit from using a double clad fiber for illumination through the single-mode core and efficient collection through the large area inner cladding. The system allows for multiplexing second harmonic generation (SHG) and two-photon excitation fluorescence (2PEF), collected from the inner cladding using a double-clad fiber coupler; and reflectance confocal microscopy (RCM), detected from the core acting as the confocal pinhole. This all-fiber system is more compact and less sensitive to alignment, but requires carefully managing the transmission of the femtosecond pulse in the fiber. Chromatic dispersion and self phase modulation (SPM) are the main effects to consider during the propagation of the pulse in fiber. Indeed, dispersion and SPM broaden the pulse both temporally and spectrally, thus drastically reducing generation of nonlinear signals. Additionally, with a source centered at 1550 nm, we benefit from reduced sample scattering thus increasing the depth of field in comparison with systems operating at 800 nm.

10069-78, Session PSun

Visualization of active ingredients uptake in seed coats with stimulated Raman scattering microscopy

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The tissues surrounding the seeds play an important role in the control of germination vigour and in the uptake of active ingredients (AIs) applied as seed dressings. Seed vigour is influenced by the coatings applied during processing. The seeds can reduce costs for growers through more efficient germination rates and have less environmental impact due to more efficient use of AIs.

In this study, we use stimulated Raman scattering (SRS) microscopy with a fiber laser source for analyzing modes of deuterium oxide (D2O, heavy water) into intact Brassica oleracea seeds and use it for understanding the chemistry of seed permeability to applied compounds. D2O has been used to investigate how seed coat composition modulates water permeation since the OD stretch (2500 cm-1) will provide a Raman signature that does not interfere with CH and OH bands of endogenous seed components. Using epi-detected SRS and two-photon microscopy to determine uptake kinetics and efficiency, we can understand the potential of seed coat manipulation for enhancing the AIs uptake. We demonstrate that SRS microscopy is an ideal tool for visualizing uptake of AIs in seed coats and worthy of further applications in real field conditions.

10069-79, Session PSun

Absorption characterization of immersion medium for multiphoton microscopy at the 1700nm window

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Larger imaging depth is the quest of almost all the imaging modalities, including multiphoton microscopy (MPM). Recently, it has been domonstrated that excitation at the 1700-nm helps extending imaging depth in MPM, optical coherence tomography, as well as photoacoustic

imaging compared with excitation at other wavelengths. In MPM, immersion objective lenses with high numerical aperture (NA) are typically used to achieve better signal resolution, higer signal collection efficiency, and stronger signal generation. Although physically short (~mm), this extra optical path length traversed by the excitation light inevitably introduces absorption of the excitation light, and as a result leads to a decrease in the signal generation. Here we demonstrate experimental characterization of absorption spectrum of various immersion media at the 1700-nm window, including water (H2O), deuterium oxide (D2O), and several brands of immersion oil. Our results identify either the best immersion medium for a specific wavelength, or the best wavelength for a specific immersion medium at the 1700-nm window. Furthermore, through quantitative MPM experiments comparing different immersion media, we show that the MPM signal levels can be enhanced by more than ten fold simply by selecting the proper immersion medium, in good agreement with theoretical expextation based on the absorption measurement. Our results will offer guidelines for signal optimization in MPM at the 1700-nm window.

10069-80, Session PSun

Moxifloxacin: Clinically compatible contrast agent for multiphoton imaging

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Multiphoton microscopy (MPM) is a nonlinear fluorescence microscopic technique widely used for cellular imaging of thick tissues and live animals in biological studies. However, MPM application to human tissues is limited by weak endogenous fluorescence in tissue and cytotoxicity of exogenous probes. Herein, we describe the applications of moxifloxacin, an FDA-approved antibiotic, as a cell labeling agent for MPM. Moxifloxacin has bright intrinsic multiphoton fluorescence, good tissue penetration and high intracellular concentration. MPM with moxifloxacin was demonstrated in various cell lines, and animal tissues of cornea, skin, small intestine and bladder. Moxifloxacin-based MP imaging technique of cellular morphology and dynamics within tissues at significantly reduced laser power compared to autofluorescence-based imaging. Clinical application is promising since imaging based on moxifloxacin labeling could be 10 times faster than imaging based on endogenous fluorescence.

10069-81, Session PSun

In situ monitoring of collagen fibers in human skin using a photonic-crystal-fiber-coupled, hand-held, second-harmonic-generation microscope

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Second-harmonic-generation (SHG) microscopy is a powerful tool for in situ monitoring of collagen fibers in human skin. However, its practical use in the dermatological field is still limited due to the bulky and complicated setup. If fiber delivery of the laser light and a compact probe head are introduced into the microscopy, its compactness, robustness, flexibility, and hence convenience, will be largely enhanced.

In this paper, we constructed a photonic-crystal-fiber-coupled, hand-held SHG microscope for in situ monitoring of collagen fibers in human skin. Fiber delivery of the 1250-nm, 80-fs pulse light from a mode-locked Cr:Forsterite laser source to SHG microscopy was achieved without the need for external chirp compensation by a large mode area photonic crystal fiber (PCF). On the other hand, SHG microscopy setup, composed of galvano mirrors, relay lenses, objective lens, dichroic mirror, optical filters, and photon-counting photomultiplier, was enclosed into a hand-held probe head (width = 310 mm, height = 150 mm, and depth = 50 mm). The combination of PCF with the compact probe head largely enhances the flexibility of the measurement site in the human skin. We compared the imaging performance between the conventional SHG microscope and the hand-held SHG microscope, and confirmed the comparable performance to each other. Finally, we demonstrated in situ visualization of collagen fibers in human skin using the hand-held SHG microscope. The constructed system will be a powerful tool for various dermatological applications.

10069-82, Session PSun

Transient absorption imaging of glycated hemoglobin in blood of diabetes patients

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Type 2 diabetes is an increasingly prevalent disease, with which more than 400 million people worldwide were diagnosed in 2016. Long-term complications of diabetes, including diabetic neuropathy and heart disease make timely diagnosis of diabetes stage indispensable. Diagnosis of diabetes based on glycated hemoglobin (HbA1c) is a most accurate procedure since HbA1c is widely regarded as a stable biomarker with little biological variability. The threshold of HbA1c among hemoglobin (Hb) is 6.5% in determining diabetes. However, current detection methods for HbA1c, such as boronate affinity chromatography, enzymatic assays, are time-consuming. Moreover, none of them provide vivid mapping of HbA1c fraction down to single red blood cell level. Here, for the first time, we demonstrate a label-free transient absorption microscopy approach to quickly differentiate HbA1c from Hb and quantify HbA1c fraction. With 10 ?s as pixel dwell time, a time-resolved stack can be yielded within 40 s. Decay constants from Hb and HbA1c were revealed to be distinctly different, which comes from different excited state dynamics. Subsequently, a linear calibration curve from standard HbA1c solutions was created through phasor analysis. Without any curve-fitting-induced errors and priori information, phasor analysis has the capability to accurately resolve the tiny difference (e.g. 2%) in HbA1c levels. This liner curve was further employed to determine the HbA1c levels of red blood cells from diabetic and healthy blood. Through this approach, our results showed a good correlation with that measured by other standard methods, which suggests that transient absorption microscopy can be applied to rapidly and accurately measure HbA1c fraction in red blood cells from diabetes patients.

10069-83, Session PSun

Quantitative evaluation of the dose-time dependences of extracellular matrix changes after gamma-irradiation by laser scanning microscopy

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Damages of extracellular matrix are known to play an important role in the development of adverse events in normal tissues after radiation exposure. The study objective was the quantitative evaluation of the dose-time dependences of extracellular matrix changes after gamma-irradiation by laser scanning microscopy with second harmonic generation and two-photon excitation imaging.

Rat's bladder was irradiated (1.25 MeV, Co60) at a single dose of 2 Gy, 10 Gy and 40 Gy by a local field (18 animals total). In a day, a week and a month after irradiation LSM-imaging of bladder slices was performed by LSM 510 Meta (Carl Zeiss, Germany). Excitation was implemented at 800 nm and a pulse repetition frequency of 80 MHz, registration at 362-415 nm (collagen) and 512-576 nm (elastin). For quantitative evaluation a mean intensity of SHG signal and TPE signal was calculated. Calculations were performed by ImageJ 1.39p (NIH, USA). All calculated indexes were normalized to a mean intensity signal of non-irradiated bladder samples.

In a day after radiation exposure a decrease of SHG signal intensity to 0.53+0.14 a.u. (10 Gy) and to 0.42+0.04 a.u (40 Gy) was observed. In a week, signal intensity remained essentially decreased (0.41+0.28 a.u. and 0.39+0.01 a.u. respectively). In a month after irradiation, we observed a retrieval to the initial signal level after 10 Gy exposure (0.93+0.32 a.u.) and a lack of recovery after 40 Gy exposure (0.38+0.07 a.u.). In case of 2 Gy exposure, there was no significant changes independently on time after irradiation. Numerical processing of LSM images provided information concerning the processes of radiation-induced changes of normal tissues in addition to visual evaluation.

10069-84, Session PSun

Multimodal imaging of vocal fold scarring in a rabbit model by multiphoton microscopy

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Vocal fold scarring as a result of injury or disease can lead to voice disorders which can significantly affect the quality of life. During the scarring process, the normally elastic tissue of the vocal fold lamina propria is replaced by a much stiffer collagen-based fibrotic tissue, which impacts the fold's ability to vibrate. Surgical removal of this tissue is often ineffective and can result in further scarring. Injectable biomaterials, a form of tissue engineering, have been proposed as a potential solution to reduce existing scars or prevent scarring altogether. In order to properly evaluate the effectiveness of these new materials, multiphoton microscopy emerges as an effective tool due to its intrinsic multiple label-free contrast mechanisms that highlight extracellular matrix elements. In this study, we quantitate the spatial distribution of collagen and elastin fibers in a rabbit model using second harmonic generation (SHG) and two photon autofluorescence (TPAF) applied to unlabeled tissue sections. In comparison to traditional methods that rely on histological staining or immunohistochemistry, SHG and TPAF provide a more reliable detection of these native proteins. The evaluation of collagen levels allows us to follow the extent of scarring, while the presence of elastin fibers is thought to be indicative of the level of healing of the injured fold. Using these imaging modalities, we characterize the outcome of injectable biomaterial treatments in order to direct future treatments for tissue engineering.

10069-85, Session PSun

Experimental study of optimum repetition rate and pulse duration for multiphoton microscopy at 1030 nm

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Multi-photon imaging (MPI) allows high resolution deep tissue imaging with less phototoxicity and photodamage than confocal microscopy. Traditionally, MPI systems use a relatively high cost titanium sapphire laser, but recently the significant increase in both available florescent molecules and compact laser systems such as fiber lasers has provided the potential for lower cost MPI systems. These new laser systems can have different repetition rates and pulse durations to titanium sapphire lasers, therefore a figure of merit (FOM = PpeakPave) was introduced, suggesting that provided the FOM remain constant, the repetition rate, average power and pulse duration could be varied with no effect on MPI signal within an optimum operating range [1]. Here we experimentally test the applicability of the FOM using a commercial fiber laser and microscope. We measure the signal produced from both fluorescein labeled beads and fixed SYTOX Green labeled mouse intestine samples as a function of average power and repetition rate between 90MHz and 2.85MHz with 350fs pulses, and as a function of average power and pulse duration from 120fs to 3ps at 45MHz repetition rate. We find that for both fluorescent markers there are operating ranges where the FOM describes our observations well, but observe operating ranges where the signal does not scale as predicted due to photodamage, microscope dispersion and photobleaching. These studies provide insights for optimizing laser systems for low cost, compact MPI microscopes.

[1] Two-photon fluorescence imaging with 30 fs laser system tunable around 1 micron. Resan B, Aviles-Espinosa R, Kurmulis S, Licea-Rodriguez J, Brunner F, Rohrbacher A, Artigas D, Loza-Alvarez P, Weingarten KJ. Opt Express. 2014 Jun 30; 22(13):1645661.

10069-86, Session PSun

In vivo metabolic imaging of mouse tumor models in response to chemotherapy

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The key aspect in analysis of carcinogenesis and tumor response to a therapy is energy metabolism. The aim of the study was to estimate energy metabolism in human cervical cancer HeLa and murine colon carcinoma CT26 tumors after chemotherapy using two-photon fluorescence microscopy with FLIM. Two-photon fluorescence and FLIM images of tumors were acquired using a multiphoton tomograph MPTflex (JenLab, Germany) equipped with TCSPC-based FLIM module (Becker&Hickl, Germany). The tissue structure was verified by two-photon fluorescence, second harmonic generation (SHG), and standard histopathology. Fluorescence lifetimes of

free and protein-bound forms of NAD(P)H and their relative contributions were monitored weekly after the therapy with drugs of different mechanisms of action. Tumors were inoculated subcutaneously, and the objective was positioned on the surface of the tumor with surgically opened skin flap. Metabolism in HeLa tumors was observed to be heterogeneous. Cancer cells in stroma-rich zones were more glycolitic than in cellular-rich areas. In CT26 tumors this effect was not detected. We found that on Day 14 the relative contribution of free NAD(P)H (?1) in CT26 tumors after chemotherapy decreased approximately by 4% in both groups with Irinotecan and Cisplatin in comparison with untreated control, indicating a shift towards more oxidative metabolism. On Day 21 ?1 value decreased in all treated groups. In summary, the combination of optical metabolic imaging with autofluorescence and SHG analysis provide novel, quantitative insights into tumor heterogeneity and response to the treatment. This work was partly supported by RSF (grant No. 14-15-00646) and the RFBR (grant No. 15-32-20324).

10069-87, Session PSun

Rapid hyperspectral, vibrationally resonant sum-frequency generation microscopy

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Vibrationally resonant sum-frequency generation (VR-SFG) microscopy enables chemically selective probing of biological structures that are commonly visualized with second-harmonic generation (SHG) microscopy. Despite its potential, SFG microscopy has traditionally been slow and impractical for biological imaging. We have developed a laser scanning VR-SFG microscope capable of generating images with acquisition rates of 1 frame/s. The system is based on a 1030 nm fiber laser and a synchronously pumped mid-infrared (MIR) optical parametric oscillator (OPO). We have implemented custom-designed optics that enable achromatic laser beam scanning from the near infrared (NIR) to the MIR, which significantly improves image quality. Using this optimized system and by tuning the OPO, we have achieved hyperspectral SFG imaging of fibrillar and micro-crystalline biological structures.

10069-89, Session PSun

The effect of changing the numerical aperture in SHG microscopy of cartilage

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Second harmonic generation (SHG) microscopy is a promising imaging technique for collagenous tissues due to its non-invasiveness and potential for 3D imaging. In tissues with densely packed and thin collagen fibrils, such as cartilage, the focal volume of the laser can comprise multiple fibrils, and the SHG from each of these fibrils can interact coherently before contributing to the detected signal. Coherent amplification is achieved when the fibrils are aligned and oriented in the same direction. The effect of changing the size of the focal volume, as determined by the numerical aperture (NA), is therefore dependent on the length scale at which the fibrils are aligned. This effect on the image contrast is important to examine experimentally before SHG can be used in the clinic. In this study, we measured the SHG intensity and radiation direction as a function of the NA of the excitation and collecting objectives in different areas of the immature articular cartilage of young pigs. The results showed that varying the NA of the excitation objective had more effect on the SHG signals detected in the forward compared to the backward direction and that this effect varied considerably between different areas of the tissue. The results clearly demonstrated that SHG imaging differs from conventional histology, and the image contrast should only be interpreted in light of its imaging conditions.

10069-90, Session PSun

Label-free imaging of cortical structures with multiphoton microscopy

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Cortical structures in the central nervous system exhibit an ordered laminar organization. Defined cell layers are significant to our understanding of brain structure and function. In this work, multiphoton microscopy (MPM) based on second harmonic generation (SHG) and two-photon excitation fluorescence (TPEF), which was applied for qualitatively imaging the structures and pathology of cerebral and cerebellar cortex from the fresh, unfixed, and unstained specimen. MPM is able to effectively identify neuron and axon in cerebral cortex, as well as glial cell, Purkinje cell, and granule cell in cerebellar cortex at subcellular resolution. In addition, we develop automated image analysis algorithms to quantify the histologic features based on the intrinsic nonlinear optical contrast. The distribution of neuron and axon in cerebral cortex, and the location of Purkinje cell layer in cerebellar cortex are quantitatively detected, further clearly visualizing the laminar structure of cerebral and cerebellar cortex. Moreover, hypercellularity, neuronal injury, and axonal density are also quantitatively characterized in MPM images. These results suggest that with the development of the feasibility of two-photon fiberscopes and microendoscope probes, the combined MPM and quantitative methodology holds potential to provide supplementary information to augment the diagnostic accuracy of neuropathology and in vivo identification of various neurological illnesses in clinic.

10069-91, Session PSun

Epi-direction detected multimodal imaging of an unstained mouse retina with a Yb-fiber laser

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Multimodal (THG, SHG, 2PEF and 3PEF) depth resolved imaging of the retina is performed using ultrashort 1060nm laser pulses. Different layers of the retina, from the pigmented laser to the ganglionic layer are identified by their morphology and the prevalent type of nonlinear optical signal detected. The use of ultrashort pulses greatly enhances third harmonic generation (THG) and three photon excited fluorescence (3PEF) while reducing the possibility of thermal damage to living tissue. Our preliminary results on intact mice, cat and dog retinas, suggest multimodal depth resolved imaging with 1060nm pulses might provide a higher resolution alternative to optical coherence imaging.

10069-92, Session PSun

In vivo three-photon activity imaging of GCaMP6-labeled neurons in deep cortex and the hippocampus of the mouse brain

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Two-photon microscopy (2PM) combined with genetically encoded calcium indicators (GECIs) has been the major method for large population recording of neuronal activity in the mouse cortex. It has been shown that 3PM extends the maximum imaging depth with the stronger suppression of out-of-focus fluorescence background and less scattering of the longer excitation wavelength. In this study, we show that 3PM with 1300nm excitation is able to image the firing GCaMP6s labeled neurons in deep cortical layers and the hippocampus that are otherwise inaccessible for 2PM with 920nm excitation, with the same microscope and mice. We first demonstrate that 3PM provides comparable signal levels as 2PM, and captures identical activity information in shallow cortical layers down to L4, with a time multiplexing approach that alternates 2P and 3P excitation sources on the microsecond time scale to achieve essentially simultaneous recording. We further compared the performance of 3PM with 2PM in deeper cortical layers L5 and L6. 3PM showed about two orders of magnitude better signal-to-background ratio (SBR) than 2PM around 750um below the pia, where 2PM approaches the depth limit for even resolving structural feature of neurons. Finally, we demonstrate 3PM activity recording in the hippocampal stratum pyramidale (SP) around 1mm deep, with more than 150 total neurons recorded within the field-of-view. Our work will extend the applications of GCaMP6 family of GECIs to unprecedented depth and enable functional imaging of large population of neurons that were previously inaccessible by 2PM.

10069-94, Session PSun

Multimodal optical imaging database from tumour brain human tissue: endogenous fluorescence from glioma, metastasis and control tissues

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Surgical resection, whenever feasible, is the first-line therapy to treat central nervous system tumors1,2. The extent of resection is a major prognostic factor whatever the histopathological subtype3. If maximal resection is required, preserving surrounding eloquent brain areas is warranted to equilibrate the onco-functional balance: improving the outcomes through maximal tumor removal and preserving the postoperative quality of life. The identification of the tumor limits, including gliomas and metastasis is difficult intraoperatively.

We aim to construct a database of multimodal optical imaging information from fresh and fixed human samples,to further enhance the specificity and sensitivity of optical analysis on brain tissues., Four different contrasts were used by our team: 1) spectral analysis, 2) two photon Fluorescence Lifetime Imaging (FLIM) and one photon time domain measurement, 3) Second Harmonic Generation (SHG) imaging and 4) Fluorescence imaging using one and two photon excitation. These series of specific multimodal "optical signatures" linked to different types of tissues will help to (i) define the best excitation and collection parameters, (ii) obtain data allowing differentiation of healthy and tumorous tissues, and (iii) give new insights into brain morphology. These results confirm the clinical relevance of this multimodal optical analysis, which can be easily applied to neurosurgical purpose for a better definition of surgical margins for gliomas, and metastasis.

This multimodal optical analysis could overcome limits of actual technics and provide clinically useful material on human brain tumors. The results on human biopsies were also compared to the gold standard histopathology.

JenLab Young Investigator Award

10069-95, Session PSun

Circularly polarized coherent anti-stokes Raman scattering microscopy as a novel tool for quantification of molecular disorganization in demyelinated axons

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Crush to the mammalian spinal cord leads to demyelination of axons followed by disruptions in their molecular architecture. Though numerous studies have shown close associations between the broken molecular ordering and functional losses of axons, there has been no reliable tool that indicates the degree of the disorder. In this paper, circularly polarized coherent anti-Stokes Raman scattering(CP-CARS) microscopy is used as a tool to quantify the molecular disorganization of myelin sheath. CP-CARS shows high sensitivity to the presence of CH2 bonds – major constituents of myelin sheath – as well as their arrangements, making it possible to quantify the symmetry of specific chemical bonds. Furthermore, the signal acquired is free of nonresonant background for highly sensitive measurements. Our experimental measurements on multi lamellar DPSC vesicles showed five times higher signal-to-noise ratio compared to conventional CARS microscopy. Our work suggests a novel method for diagnosis of spinal cord injury in label-free manner.

10069-96, Session PSun

Imaging of epithelial-mesenchymal transition process of live gastric cancer cells with spectral focusing based hyperspectral stimulated Raman scattering

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The epithelial-mesenchymal transition (EMT) process is an important step towards the metastasis of cancer cells. Study of the EMT process is clinically valuable for the research on the cancer development and the diagnosis of cancer cells before the metastasis occurs. In this paper, we report the development of a spectral focusing based hyperspectral stimulated Raman scattering (SRS) imaging technique for label-free imaging of the biochemical/biomolecular and morphological changes in live gastric cancer cells during the epithelial-mesenchymal transition (EMT) process. The developed SRS imaging technique can cover the 2800-3100 cm-1 region in ~10 secs by scanning the time delay between the chirped pump and Stokes beams. The distributions of proteins, lipids and DNA can be extracted from the hyperspectral SRS images using multivariate curve resolution (MCR) method. The gastric cancer cells become fibroblast-like shape 8 hours after the initiation EMT process. The contents of proteins and lipids start to increase after 4 hours of EMT process and continue to increase by a factor of three after 12 hours. Our results show that the hyperspectral SRS imaging technique is a powerful label-free imaging method for visualizing the

morphological and biological/biochemical change during the EMT process and other biological activities of live cells

10069-97, Session PSun

Spatial frequency modulated imaging (SPIFI) with amplitude or phase grating from a spatial light modulator

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Spatial Frequency Modulated Imaging (SPIFI) with single element detection has previously been demonstrated with a time varying amplitude spatial frequency. This has been shown in a variety of modalities (linear, TPEF, SHG) and also with variations on the base design to provide additional dimensions of information. SPIFI is also capable of providing enhanced resolution images. However, the signal-to-noise is a limiting factor in the quality of the resolution enhancement. A significant contributor to poor signal to noise for enhanced resolution images is the 0th order of diffracted light from the time-varying spatial frequency. We present a microscope design which uses a nematic spatial light modulator to provide a time varying amplitude or phase grating. The peak-to-peak phase difference (excursion) allows for control of power distribution into the diffracted orders. This in turn allows for an improved signal-to-noise ratio in the image and in the enhanced-resolution images which SPIFI provides. Twophoton excitation fluorescence images at two different excursion values of 10-um fluorescent polystyrene beads are presented. Additionally, the microscope can provide spatial gratings in polarization which provide an alternative means of imaging in third harmonic generation (THG). THG images are provided using amplitude, phase and polarization gratings.

JenLab Young Investigator Award

10069-98, Session PSun

Nonlinear optical Stokes ellipsometry for pixel-by-pixel SHG polarization analysis of tissues

Garth J. Simpson, Ximeng Dow, Emma Kerian, Shane Sullivan, Paul Schmitt, Purdue Univ. (United States)

Polarization-dependent SHG microscopy of tissue is highly sensitive to collagen structural changes associated with differences in disease states. However, the most common polarization-dependent measurements based on waveplate manipulations typically introduce 1/f noise that limits the fidelity of the subsequent analysis. A general mathematical framework is described that connects both n high-speed phase modulation measurements and more conventional approaches based on polarization rotation within a single coherent architecture. In this framework, the local-frame tensor at each SHG-active position within the field of view can be independently determined. In combination with quantum chemical hyperpolarizability calculations of the collagen triple helix, these local-frame tensor elements are directly connected back to molecular-level collagen structure. Interestingly, the polarization analysis reveals the presence of a significant fraction of broadly distributed anti-parallel collagen within individual fibers. In addition to this molecular-level information, the polarization analysis simultaneously recovers both the azimuthal and polar fiber orientation angles at each pixel. These orientation angles can in turn aid in microstructural analysis of collagen frameworks. The combination of molecular-level and microstructural information has the potential to significantly improve the information content accessible from SHG measurements of tissue samples in disease diagnosis.

10069-99, Session PSun

Differential levels of metabolic activity in isolated versus confluent/partially confluent HeLa cells are analyzed by autofluorescent NAD(P)H using multi-photon FLIM microscopy

Andrea Chandler, Palo Alto High School (United States); Aaron Chandler, Univ. of California, Davis (United States); Horst K. Wallrabe, Ammasi Periasamy, Univ. of Virginia (United States)

NAD(P)H is a known biomarker for cellular metabolism; a higher ratio of enzyme-bound NAD(P)H to free/unbound NAD(P)H indicates an increase in metabolic activity. Free NADH has a shorter fluorescence lifetime (?1), the bound version (?2) a longer lifetime. FLIM's unique capability to establish inter alia the relative fractions of ?1 (a1%) and ?2 (a2%) in each pixel, determines the level of metabolic activity. The relative abundances of bound NAD(P)H were analyzed for single cells, confluent and partially confluent cells within 3 Fields-of-View (FoVs). A gradient of increasing a2% levels of bound NAD(P)H from single, partially confluent to confluent cells was observed.

10069-101, Session PSun

Multimodal imaging of optical biomarkers for label-free oral cancer diagnosis

Arunthathi Manickavasagam, King's College London (United Kingdom); Richard Cook, Frederic Festy, King's College London (United Kingdom)

Oral cancer is one of the ten most common malignancies worldwide, associated with high recurrence and poor survival rate. The current approaches to screening and identification of oral neoplasia relies on subjective interpretations, resulting in low sensitivity and specificity. Definitive cancer diagnosis helps to improve patient survival by reducing the need for multiple biopsies, thereby alleviating patient trauma and minimising the diagnostic lead times. This mandates specific and sensitive detection and discrimination of both benign and malignant lesions aiding provision of optimal prognosis.

The aim of this study was to develop a multimodal optical diagnostic tool, combining Raman spectroscopy, wide-field fluorescence (WF) imaging, second-harmonic generation (SHG) imaging, two-photon fluorescence intensity (2PF) and life-time imaging (FLIM) for label-free tissue characterisation. The proposed system quantitatively performs objective classification of normal, benign and cancerous lesions without selective sampling the areas to be imaged, yielding improved sensitivity and specificity compared to current techniques.

The pre-processed Raman, WF, 2PF, SHG and FLIM images were co-registered and background segmented. An in-house statistical method was used to perform binary and multiclass classification of the normal, benign and malignant tissue types. The neoplastic and non-neoplastic tissues were discriminated with 100% sensitivity and 100% specificity. In multiclass classification, the benign was detected with 100% sensitivity and 95.2% specificity and the malignant was classified with 100% sensitivity and 71.4% specificity.

In summary, an optical diagnostic model for unbiased classification of normal, benign and malignant lesions was developed. This multi-modal imaging approach showed the potential to serve as a reliable screening tool for pathologists to objectively identify the diseased cases.

10069-21, Session 5

SRS microscopy: The quest for sensitivity *(Invited Paper)*

Xiaoliang S. Xie, Harvard Univ. (United States)

In less than eight years, stimulated Raman scattering (SRS) microscopy has been widely applied to biology and medicine, given its high specificity and sensitivity for rapid label-free imaging. The current challenge is to further increase its sensitivity in order to detect many important molecules whose spatial distributions have not been mapped out within living organisms. SRS's sensitivity, which is limited by the shot noise of lasers, will be discussed in the context of relevant biomedical applications.

10069-22, Session 5

Hyperspectral stimulated Raman scattering imaging facilitates accurate diagnosis of human prostate cancer *(Invited Paper)*

Shuhua Yue, Sishan Cui, BeiHang Univ. (China); Ping Wang, Huazhong Univ. of Science and Technology (China)

Most prostate cancers (PCa) are slowly growing, and only the aggressive ones require early diagnosis and effective treatment. The current standard for PCa diagnosis remains histopathology. Nonetheless, for the differentiation between Gleason score 6 (low-risk PCa), which can be left without treatment, and Gleason score 7 (high-risk PCa), which requires active treatment, the inter-observer discordance can be up to 40%. Our previous study reveals that cholesteryl ester (CE) accumulation induced by PI3K/AKT activation underlies human PCa aggressiveness. However, Raman spectromicroscopy used in this study could only provide compositional information of certain lipid droplets (LDs) selected by the observer, which overlooked cell-to-cell variation and hindered translation to accurate automated diagnosis. Here, we demonstrated quantitative mapping of CE level in human prostate tissues using hyperspectral stimulated Raman scattering (SRS) microscopy that renders compositional information for every pixel in the image. Specifically, hundreds of SRS images at Raman shift between 1620-1800 cm-1 were taken, and multivariate curve resolution algorism was used to retrieve concentration images of acyl C=C bond, sterol C=C bond, and ester C=O bond. Given that the ratio between images of sterol C=C and ester C=O (sterol C=C/C=O) is nonlinearly proportional to CE percentage out of total lipid, we were able to quantitatively map CE level. Our data showed that CE level was significantly greater in high Gleason grade compared to low Gleason grade, and could be a factor that significantly contributed to cancer recurrence. Our study provides an opportunity towards more accurate PCa diagnosis and prediction of aggressiveness.

10069-23, Session 5

Label-free longitudinal monitoring of melanogenesis in the evolution of melanoma treatment resistance

Sam Osseiran, Hequn Wang, Massachusetts General Hospital (United States); Ken Dutton-Regester, Levi A. Garraway, Dana-Farber Cancer Institute (United States); Conor L. Evans, Massachusetts General Hospital (United States)

While melanoma is not the most common form of skin cancer, it represents the vast majority of skin cancer-related deaths. Indeed, while combination therapies such as Dabrafenib and Trametinib have shown great promise in clinical trials for treating metastatic disease, some melanoma subtypes nevertheless develop resistances to front-line treatments. Under in vitro conditions, some metastatic human melanoma cell lines have been observed to evolve resistance to treatment while simultaneously changing color under brightfield microscopy, hinting at perturbations in pigment synthesis. The process known as melanogenesis gives rise to the two forms of melanin found in mammals: eumelanin, a dark brown/black pigment, and pheomelanin, a much more pale red/blond pigment. Interestingly, pheomelanin has been shown to contribute to the onset and development of melanoma in an ultraviolet-radiation-independent manner through a mechanism of oxidative stress. Eumelanin, on the other hand, is a known antioxidant whose chemical properties seem to shield cells against oxidative damage. To study these pigments in closer detail, nonlinear optical microscopy including coherent anti-Stokes Raman scattering (CARS) was used for the specific visualization and quantification of the relative abundance of pheomelanin and eumelanin within these treatment resistant cell lines. These microscopy toolkits provide a means to monitor changes in pigmentation in a noninvasive and non-destructive manner without the use of exogenous dyes to better understand the molecular basis of treatment resistance.

10069-24, Session 5

Multimodal nonlinear optical imaging of cartilage development in mouse model

Sicong He, Hong Kong Univ. of Science and Technology (Hong Kong, China); WenQian Xue, The Univ. of Hong Kong (Hong Kong, China); QIQI SUN, XUESONG LI, HKUST (Hong Kong, China); JIANDONG HUANG, HKU (Hong Kong, China); JIANAN QU, HKUST (Hong Kong, China)

Kinesins are a group of motor proteins responsible for directional transport along microtubules. As the most abundant kinesin motor, kinesin-1 plays a significant role in the intracellular transportation and has been studied in a variety of tissues. However, its roles in cartilage and chondrocytes are not clear. In this study, a kinesin-1 heavy chain (Kif5b) knockout mouse model is used to investigate the functions of kinesin-1 in the cartilage development. We developed a multimodal nonlinear optical (NLO) microscope system integrating stimulated Raman scattering (SRS), second harmonic generation (SHG) and two-photon excited fluorescence (TPEF) to study the morphological and biomedical characteristics of fresh tibial cartilage from normal and knockout mice at different developmental stages. The combined forward and backward SHG imaging resolved the structure of collagen fibrils in the extracellular matrix of cartilage. Meanwhile, the chondrocyte morphology in distinct zones of cartilage was visualized by label-free SRS and TPEF images. The results show that the fibrillar collagen in the superficial zone of cartilage in postnatal day 15 (P15) knockout mice was significantly less than that of wild-type mice. Moreover, we observed distorted morphology and disorganization of columnar arrangement of chondrocytes in the growth plate cartilage of P10 and P15 knockout mice. Additionally, accumulation of intracellular collage was revealed in the morphologically abnormal chondrocytes of P15 knockout mice, which was not found in the control group. The appearance of intracellular collagen suggests the dysfunction of collagen secretion in Kif5b deficient chondrocytes and reveals the roles of kinesin-1 in collagen formation and chondrocyte morphogenesis.

10069-25, Session 7

Application of broadband coherent Raman imaging to histopathology *(Invited Paper)*

Marcus T. Cicerone, Charles H. Camp Jr., National Institute of Standards and Technology (United States)

We will report on application of broadband coherent anti-Stokes Raman scattering microscopy1 to chemical mapping and characterization of

resected prostate sections. While incidence of prostate cancer is very high, only a small fraction of prostate tumors will progress to advanced, metastatic disease and become dangerous, but prostatectomy and follow-on treatment have many undesirable potential side effects. Thus, it is important to predict which tumors will progress and which should be removed, but there is currently no highly reliable way to make such predictions. We will present a retrospective coherent Raman imaging study resected prostate sections focusing on locating tissue regions that present the highest diagnostic value with respect to lethal vs indolent disease. We intend that this provide a guide to optimal spectral sampling of these tissues to address this important clinical problem.

10069-26, Session 7

Stimulated Raman scattering imaging of tumor metabolism *(Invited Paper)*

Lingyan Shi, Luyuan Zhang, Yihui Shen, Lu Wei, Wei Min, Columbia Univ. (United States)

Enhanced syntheses of new biomolecules in cancer cells are the major biological processes in tumor. “Visualization and understanding of the in vivo metabolic heterogeneity within intact tumors is always a challenging question. Tumor’s anatomical and structural information is accessible by clinically-available MRI or CT. And its metabolic activity of a radioactive compound inside the body can be imaged by PET. However, these methods provide poor spatial resolution images with millimeter scale which cannot allow us to observe the biomolecules formation and heterogeneity with subcellular resolution in tumor. To demystify the new synthesized biomolecules’ spatial distribution in the tumor metabolism, we applied stimulated Raman scattering (SRS) microscopy, coupled with metabolic incorporation of isotope-labeled metabolites (such as deuterium labeled (d-) amino acids, d-glucose, d-fatty acids, d-choline) to visualize nascent biomolecules. We imaged newly synthesized biomolecules in tumors after in vivo delivery of isotope labeled metabolites into rodents. Our results generate spatial maps of the quantitative ratio between new and total proteomes and lipids and display the analysis of infiltrative tumor margins in a new point of view. This method opens a door to study tumor metabolic heterogeneity in vivo, and to observe nutrient availability in different regions, stromal and inflammatory cells’ local effects, and cell-autonomous effects regulated by clonal expansion of mutants with diffraction limited resolution.

10069-27, Session 7

Label-free biomolecular characterization of human breast cancer tissue with stimulated Raman scattering (SRS) spectral imaging

Fa-Ke F. Lu, David Calligaris, Brigham and Women’s Hospital, Harvard Medical School (United States); Yuanzhen Suo, Shanghai Jiao Tong Univ. (China); Sandro Santagata, Alexandra J. Golby M.D., Brigham and Women’s Hospital, Harvard Medical School (United States); X. Sunney Xie, Harvard Univ. (United States); Melissa A. Mallory, Mehra Golshan, Deborah A. Dillon, Nathalie Y. R. Agar, Brigham and Women’s Hospital, Harvard Medical School (United States)

Stimulated Raman scattering (SRS) microscopy has been used for rapid label-free imaging of various biomolecules and drugs in living cells and tissues (Science, doi:10.1126/science.aaa8870). Our recent work has demonstrated that lipid and protein mapping of cancer tissue renders pathology-like images, providing essential histopathological information with subcellular resolution of the entire specimen (Cancer Research, doi: 10.1158/0008-5472.CAN-16-027). We have also established the first SRS imaging Atlas of human brain tumors (Harvard Dataverse, doi: (doi:10.7910/DVN/EZW4EK). SRS imaging of tissue could provide invaluable information for cancer diagnosis and surgical guidance in two aspects: rapid surgical pathology and quantitative biomolecular characterization. In this work, we present the use of SRS microscopy for characterization of a few essential biomolecules in breast cancer. Human breast cancer tissue specimens at the tumor core, tumor margin and normal area (5 cm away from the tumor) from surgical cases will be imaged with SRS at multiple Raman shifts, including the peaks for lipid, protein, blood (absorption), collagen, microcalcification (calcium phosphates and calcium oxalate) and carotenoids. Most of these Raman shifts have relatively strong Raman cross sections, which ensures high-quality and fast imaging. This proof-of-principle study is sought to demonstrate the feasibility and potential of SRS imaging for ambient diagnosis and surgical guidance of breast cancer.

10069-28, Session 7

Probing antimicrobial susceptibility in a single fungus by SRS microscopy

Caroline Karanja, Weili Hong, Waleed Younis, Ji-Xin Cheng, Mohamed Seleem, Purdue Univ. (United States)

Candida is the single most important cause of fungal bloodstream infections worldwide causing significant mortality as high as 50%. This high mortality rate is, in part, due to the inability to rapidly diagnose and simultaneously initiate an effective antifungal therapy early in the disease process. Current culture-based diagnostics are often slow, requiring several days to complete, and are only 50% sensitive in diagnosing candidemia (Candida bloodstream infection). For every 12 hours of delay in starting correct antifungal therapy, the risk of death for a given patient with candidemia increases by 200%. To address this unmet need, we explored the potential of employing stimulated Raman Scattering (SRS) imaging to diagnose candidemia and probe metabolic differences between resistant and susceptible strain at a single cell level. Metabolism is integral to pathogenicity; microorganism have very short life cycles, and therefore only a few hours are needed to observe a full metabolic cycle. SRS imaging at C-H vibration frequency at 2850 cm-1 revealed a substantial difference in lipogenesis between the susceptible and resistant C. albicans. Treating the C. albicans with fluconazole, an antimicrobial drug that targets ergosterol biosynthesis only affected the lipogenesis in the susceptible strain. Our results show that single-cell metabolic imaging under a SRS microscope can be used for diagnose candidemia and early detection of antimicrobial susceptibility.

10069-29, Session 7

New approaches to intracellular imaging by stimulated Raman scattering microscopy

Martin Lee, William Tipping, The Univ. of Edinburgh (United Kingdom)

Visualising the complex interplay between bioactive small molecules and an intricate network of cellular machinery represents a major challenge within chemical biology, medical sciences and pharmaceutical development. Herein, we describe a study of the design, synthesis, structure-activity relationship and biological impact of a range of alkyne derived Raman markers as a toolbox approach for Stimulated Raman Scattering Imaging. A range of proposed tags are examined in-silico for activity and then synthesised generating a library of analogues of the natural product, Anisomycin. Specifically, we report the use of bisaryl butadiyne-anisomycin (BADY-anisomycin) in intracellular SRS microscopy studies of uptake, trafficking and localisation within live and fixed cells. Following rational design and synthesis, BADY-anisomycin was shown to produce an intense Raman band at 2219 cm-1, that is centrally located within the cellular silent region and is approximately 60 times more Raman active than the

corresponding propargyl-anisomycin. SRS imaging is presented in tandem with confocal fluorescence microscopy showed rapid uptake of BADY-anisomycin and confirmed enrichment in the endoplasmic reticulum. Finally, we demonstrate two-colour imaging utilising EdU, an alkyne-containing proliferation probe and BADY-aniosmycin. These studies harbinger the potential of SRS microscopy in tandem with bespoke Raman labelling campaigns for live-cell, multi-colour intracellular imaging.

10069-30, Session 7

Imaging living brain cells with stimulated Raman scattering microscopy

Miriam J. B. Moester, Judith H. van Santen, Ruud Toonen, Freek Ariese, Johannes F. de Boer, Vrije Univ. Amsterdam (Netherlands)

Stimulated Raman Scattering (SRS) microscopy provides a powerful label-free research tool for investigating biomolecules in living cells and organisms. We show the potential of SRS microscopy for imaging dynamics in living brain cells.

In SRS, energy is transferred from one color laser beam to another, provided the energy difference between them matches a molecular vibration of a molecule in focus. By applying amplitude modulation to one of the beams, modulation transfer can be measured. Combined with laser scanning microscopy, this technique allows for fast and sensitive imaging with sub-micrometer resolution. It allows imaging the distribution of a specific Raman vibration throughout the volume of the cell. In practice, many compounds in the cell have overlapping Raman spectra. By employing stable isotope labeling, specific compounds can be targeted and visualized independently. Unlike fluorescent labeling or electrophysiological techniques, these bio-orthogonal tags allow investigation of undisturbed biological processes in the cell.

Our set-up is optimized for single wavelength SRS imaging at high sensitivity with shot noise limited detection. SRS microscopy provides a powerful platform for imaging the processes of production, storage and transport in brain cells.

10069-31, Session 8

Surface-enhanced stimulated Raman scattering in the single molecule limit *(Invited Paper)*

Eric O. Potma, Kevin T. Crampton, Alexander Fast, Vartkess A. Apkarian, Univ. of California, Irvine (United States)

We present combined surface-enhanced stimulated Raman scattering (SE-SRS) and surface-enhanced coherent anti-Stokes Raman scattering (SE-CARS) measurements on individual plasmonic antennas dressed with bipyridyl-ethylene molecules. By carefully optimizing the conditions for performing SE-SRS experiments, we have obtained stable and reproducible molecular surface-enhanced SRS spectra from single nano-antennas. Using surface-enhanced Raman scattering (SERS) and transmission electron microscopy of the same antennas, we confirm that the observed SE-SRS signals originate from only one or a few molecules. We highlight the physics of surface enhancement in the context of coherent Raman scattering and derive sensitivity parameters under the relevant conditions. The implications of single molecule SRS measurements are discussed.

10069-32, Session 8

3D chirped CARS microspectroscopy for biological objects visualization

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Chemically selective imaging of complex biological objects is of high interest nowadays. The approaches based on multiphoton fluorescence and Raman microscopy are widely exploited. Possessing a whole series of merits CARS microscopy systems are rather complex. Recently, in most cases picosecond laser pulses are used for such systems. Meanwhile, combination of picosecond and femtosecond pulses can bring additional features which stem from spectral characteristics of laser pulses in femtosecond time scale. In this work, we study chirped CARS (c-CARS) two pulse variation of CARS and its potential applicability for biological systems. Pump femtosecond pulse in our c-CARS scheme is stretched up to 10-15 ps while Stokes femtosecond pulse can scan pump frequencies for different delays between pulses. This delay probing allows to reconstruct IR spectrum much faster than in conventional CARS-microscopes thus can be used for 3D scanning systems where time parameter is crucial. Moreover, targeting of IR frequency range from “fingerprints” region to ~4000 1/cm is possible due to fast wavelength tuning of the pump pulse. For several simple samples we also clarify spectral resolution of the system as well as firm separation of resonance and non-resonance CARS signals. Our fast 3D scanning system is based on two sets of galvanic mirrors.

This work was supported by the Russian Science Foundation grant 14-14-00856.

10069-33, Session 8

Cascade CARS Microscopy by sub 30 fs laser pulses

Vitor Pelegati, Bernardo B. C. Kyotoku, Lazaro A. Padilha, Carlos Lenz Cesar, Univ. Estadual de Campinas - Instituto de Física “Gleb Wataghin” (Brazil)

We used six-wave mixing CARS process, which involves three photons of the pump beam and two of the Stokes beam to acquire images of a fresh mouse ear tissue sample. The signal comes from the Parallel cascade CARS, a process where the generate photon of the first CARS process leads to a virtual transition from the excited vibration level in a second CARS process. It has a dependency on the fourth power of chi-3, which means it will show a better contrast between resonant vs non-resonant signals. The pump beam was generated by a NOPA at 800 nm with sub 30 fs and with 200 kHz repetition rate, while the Stokes beam was at 1040 nm with 350 fs, which is the residual from the same beam employed for pumping the NOPA. We acquired images for the 2847 cm-1 CH2 symmetric stretching vibration level with a 10X NA 0.3 objective, mouse ear will show strong resonance due to its high lipid content cells, we also measured the spectrally integrated signal as a function of pump wavelength to observe resonance dependency on ethanol. The NOPA have a wide bandwidth, ~60 nm, which will generate strong non-resonant signal for CARS, but the cascade CARS shows significantly less non-resonant signal. The images demonstrate that cascade CARS by a sub 30 fs pulse shows better contrast ratio than CARS.

10069-34, Session 8

Nonresonant background suppression for coherent anti-Stokes Raman scattering microscopy using multi-wavelength time-lens sources

Bo Li, Kriti Charan, School of Applied and Engineering Physics, Cornell Univ. (United States); Ke Wang, College of Optoelectronic Engineering, Shenzhen Univ. (China); David

Sinefeld, Chris Xu, School of Applied and Engineering Physics, Cornell Univ. (United States)

Coherent anti-Stokes Raman scattering (CARS) allows label-free imaging of biological samples with endogenous image contrast based on vibrational spectroscopy. Despite these advantages, a major drawback of CARS is the existence of a nonresonant background, which obscures the contrast of the resonant CARS image. In practice, an image free of non-resonant background can be acquired by subtracting the off-resonance image from the on-resonance one, however, the on- and off-resonance images are typically acquired by tuning the wavelength of the Stokes laser, and the slow tuning speed inevitably introduces spectral artifacts due to sample motion in live cell or tissue imaging. This problem can be solved by using three synchronized lasers, which allow the simultaneous recording of on- and off-resonance images. Current three-color synchronized lasers for CARS imaging are realized by OPOs or synchronized mode-locked Ti:Sapphire laser, which are costly and difficult to implement in practice. We demonstrate a robust, cost-effective, all-fiber, two-wavelength time-lens source for background-free CARS imaging. The time-lens source generates two picosecond pulse trains simultaneously: one fixed at 1064 nm and the other tunable between 1040 nm and 1075 nm. The average power for each wavelength is approximately 400 mW. When synchronized to a mode-locked Ti:Sapphire laser, the two wavelengths are used to obtain on- and off-resonance CARS images. Real-time (pixel by pixel) subtraction of the nonresonant background in the CARS image is achieved by the synchronization of the microscope pixel clock and the time-lens source. Background-free CARS imaging of sebaceous glands in ex vivo mouse tissue is demonstrated.

10069-35, Session 9

CARS microscope enables BISTRO measurements *(Invited Paper)*

Charles W. Ballmann, Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Raman and Brillouin microscopy are emerging techniques for non-invasive biomedical imaging. While Raman spectroscopy provides a chemical contrast imaging, Brillouin spectroscopy allows non-contact assessment of local viscoelastic properties. We report for the first time a nonlinear Raman microscope based on coherent anti-Stokes Raman scattering (CARS), which simultaneously allows Brilloion Imaging and Sensing via Time Resolved Optical (BISTRO) measurements. In our presentation, we will describe our approach and the developed instrument and will illustrate its performance using several different tissue samples.

10069-36, Session 9

Wide-field surface-enhanced CARS microscopy of cells

Alexander Fast, John T. Kenison, Eric O. Potma, Univ. of California, Irvine (United States)

We have previously demonstrated a total internal reflection, wide-field CARS microscope, where the signal is enhanced with the aid of a thin gold layer that supports surface plasmon polariton resonances. This surface-enhanced CARS microscope is capable of generating images of lipid structures in close proximity (<100 nm) to the glass substrate at excitation densities that are 4 orders of magnitude lower than in point-scanning CARS microscopy. In this contribution, we demonstrate its application to visualizing lipids in aqueous media, including imaging of cells, with a unique surface-sensitive contrast that cannot be obtained with conventional CARS microscopy.

10069-37, Session 9

Stimulated Raman spectroscopic imaging by microsecond delay-line tuning

Chien-Sheng Liao, Kai-Chih Huang, Weili Hong, Andy J. Chen, Caroline Karanja, Pu Wang, Gregory Eakins, Ji-Xin Cheng, Purdue Univ. (United States)

Coherent Raman imaging techniques, including coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS), are powerful tools to visualize the spatial distribution of molecules in cells or tissues. To resolve overlapped Raman bands in biological samples, there has been a great effort in developing spectroscopic coherent Raman imaging technology. Spectral scanning of a narrowband laser pulse and collection of images at a series of Raman shifts has been demonstrated to reach real-time imaging speed. However, the limited temporal resolution of each spectroscopic stack on second scale tends to distort spectral profiles from highly dynamic organelles in live cells or animals. In this work, we demonstrate stimulated Raman spectroscopic imaging using a lab-built microsecond delay tuner. By directing the collimated light to the edge of a tilted resonant mirror with a few kHz central frequency, and focusing the reflected light with a lens on a flat mirror, the retroreflected light experienced a millimeter-scale difference in optical path when the resonant mirror was scanned in one cycle of tens of microseconds. By synchronization of the resonant mirror with galvo-mirrors used for imaging scan, we collected an SRS spectrum, covering ~200 cm-1 determined by the excitation pulse within 83 ?s at each pixel of an image. The speed advantage allows chemical imaging of highly dynamic systems and 3-D mapping of fat storage in live C. elegans. Collectively, the presented efforts pushes Raman spectroscopy that is conventionally used for chemical imaging of fixed specimens towards compositional mapping of live intracellular compartments.

10069-38, Session 9

Sparsely-sampled hyperspectral coherent Raman scattering microscopy

Haonan Lin, Chien-Sheng Liao, Kai-Chih Huang, Pu Wang, Nan Kong, Ji-Xin Cheng, Purdue Univ. (United States)

Hyperspectral coherent Raman scattering (CRS) microscopy is an emerging non-linear optical modality enabling label-free chemical components segmentation without prior knowledge, which has seen huge potential in biology and medicine. Current acquisition speed for hyperspectral CRS is 3 seconds per data cube, which is insufficient for imaging freely moving subjects without motion artifacts. Nevertheless, increasing the image acquisition speed by reducing the signal integration time is impractical, because the signal-to-noise ratio will decrease. Alternatively, we exploited the information redundancy of spatially and spectrally adjacent pixels in hyperspectral images, so as to improve the image acquisition speed by reducing the sampled pixels in the hyperspectral data cube. Specifically, a 3D triangular wave Lissajous trajectory with high least common multiplier was used for sparse sampling, such that limited sampled pixels are uniformly scattered within the hyperspectral data cube. A model-based image in-painting algorithm was applied to recover the complete hyperspectral image. Simulations based on a hyperspectral CRS of polystyrene and poly(methyl methacrylate) beads suggest that with a properly selected sparse sampling trajectory, a sampling fill rate as low as 10% is sufficient to generate hyperspectral segmentation maps that are slightly distorted, and a sampling rate higher than 30% does not further improve the segmentation maps' quality. This work clearly demonstrates the information redundancy of hyperspectral images and shed light on the design of video-rate hyperspectral CRS imaging platform.

10069-39, Session 9

Unsupervised, quantitative analysis of coherent Raman imagery

Charles H. Camp Jr., Marcus T. Cicerone, National Institute of Standards and Technology (United States)

Recent advances in coherent Raman spectral imaging enable acquisition rates from 100's to 10,000's of spectra per second with spectral breadths comparable to traditional spontaneous Raman spectroscopy. These developments afford exploration of the chemical landscape of materials, cells, and tissues with unprecedented clarity. To take full advantage of the rich chemical data requires hyperspectral processing and analysis methods tailored to coherent Raman imagery (CRI), which are currently limited in availability. Our work addresses several fundamental challenges in CRI: conditioning and preprocessing of CRI hyperspectral cubes in an unsupervised and reliable manner, spectral unmixing to determine pure species spectral components, and finally the quantification and classification of pure species spectra with known molecular species. This final topic also reveals optimal sets of spectral components to probe with narrowband methods to maximize biomolecule species discrimination with a minimal number of laser tunings. With respect to CRI pre-processing, we will present an unsupervised pipeline that denoises hyperspectral imagery (using spatial and spectral information) and extracts the Raman peaks from raw CRI spectra using the Kramers-Kronig relationship with phase- and amplitude-error correction. With respect to spectral unmixing, we will detail our recent development of of vector flow vertex component analysis, which combines optimization methods and geometric methods to calculate the coherent Raman spectra of pure chemical species in highly-mixed images in which no pixels contain pure spectra. Finally, we will present out development of an open-source database that enables confidence in spectral assignments and the identification of defining Raman peaks.

10069-40, Session 9

A flexible and rapid frequency selective scheme for SRS microscopy

Jingting Li, Yuankai Yue, Wei-Chuan Shih, Univ. of Houston (United States)

Raman scattering is a form of vibrational scattering where incident photons interact with molecular vibration. By measuring the scattered photons at multiple wavelengths, a photon energy diagram can be generated and is known as Raman spectroscopy. When the Raman scattering process is stimulated, a form of stimulated scattering akin to the lasing process can occur. Like spontaneous Raman scattering, Stimulated Raman scattering (SRS) is a label-free imaging technique suitable for studying biological systems. Due to stimulated nature by ultrafast laser pulses, SRS microscopy has the advantage of significantly higher sensitivity, and much higher imaging speed compared to spontaneous Raman microscopy, but often at the expense of reduced spectroscopic information. SRS microscopy also has the advantage of background-free internal contrast compared to other coherent Raman techniques such as Coherent anti-Stokes Raman scattering (CARS). In this paper, we present a newly constructed femtosecond SRS microscope with a high-speed dynamic micromirror device (DMD)-based pulse shaper to achieve flexible and rapid frequency selection within the C-H stretch region near 2700 to 3000 cm-1 with spectral width of 30 cm-1 for both the pump and Stokes beam. To obtain a hyperspectral image, a line pattern was scanned across the DMD for spectral multiplexing, while a galvo scanner projects the laser spot across a 2-D plane for spatial scan, and stimulated Raman loss data was collected at each pattern. This technique is applicable to lipid profiling of biological material such as cell activity mapping, lipid distribution mapping and distinction among subclasses.

10069-41, Session 9

Ultra-broadband coherent anti-Stokes Raman scattering microscopy with a dynamically power-tuned Stokes supercontinuum

Jeremy G. Porquez, Emily R. Korfanty, Aaron D. Slepkov, Trent Univ. (Canada)

Coherent anti-Stokes Raman scattering (CARS) hypermicroscopy is a popular label-free biomedical imaging technique. CARS hypermicroscopy traditionally requires two pulsed laser sources to generate the pump/probe and the Stokes beams; however, this is often a technically challenging, and expensive technique to implement. Using a supercontinuum generating (SCG) photonic crystal fiber (PCF) instead of a standalone laser source to generate the Stokes has been shown to simplify and significantly reduce the cost of experimental setups. However, current PCFs are known to generate sufficiently intense supercontinuum only in select wavelength regions, limiting their usefulness in traditional nonlinear optical microscopy systems that utilize Ti:Sapph laser sources.

By performing a pump/probe delay-synchronized power-tuning of an SCG module to generate spectrally-engineered Stokes beams—an approach we call 'spectral surfing'—we demonstrate spectral-focusing-based multimodal CARS hypermicroscopy, capable of probing molecular vibrations from 350 cm-1 up to 4000 cm-1 in a single scan window. This represents the broadest CARS/SRS frequency range reported in a single scan.[1] Furthermore, this is achieved using a single 200-fs oscillator, operating at a fixed wavelength of 800 nm, thereby obviating the need for either an ultrashort (i.e. sub 25 fs), tunable, or multiple laser system. Our experimental approach thus significantly reduces the cost and simplifies the addition of CARS hypermicroscopy to a wide range of existing nonlinear optical imaging systems.

10069-42, Session 10

Online-FLIM at 10 images per second *(Invited Paper)*

Wolfgang Becker, Stefan Smietana, Alexander Jelzow, Becker & Hickl GmbH (Germany)

We report on a technique that displays fluorescence lifetime images at a rate of 10 images per second. Data acquisition is based on multi-dimensional TCSPC in combination with confocal or two-photon laser scanning. The image are calculated from the TCSPC FLIM data via the first moment of the decay data in the pixels of the images. The first-moment technique combines near-ideal photon efficiency with calculation times shorter than the frame times of of the commonly used galvanometer scanners. The image rate is thus not slowed down by the lifetime calculation process. Potential applications are clinical FLIM, where suspicious areas can be identified for subsequent high-accuracy imaging. In standard FLIM microscopy, the technique it helps the user select interesting cells in a large field of view for detailed analysis with high magnification and longer acquisition time.

10069-44, Session 10

Fluorescence lifetime FRET imaging of receptor-ligand complexes in tumor cells in vitro and in vivo *(Invited Paper)*

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To guide the development of targeted therapies with improved efficacy and accelerated clinical acceptance, novel imaging methodologies need to be established. Current in vivo non-invasive optical imaging techniques are limited to monitoring only the pooling of the labeled-drug at the pathologic site and cannot distinguish between co-localization and actual ligand-receptor engagement. Herein, Fluorescence lifetime Förster Resonance Energy Transfer (FLIM-FRET) imaging enables the non-invasive quantification of the level of transferrin (Tf)-transferrin receptor (TfR) ligand-receptor engagement in live subjects. Elevated FRET donor fraction (FD%) for holo-Tf (iron-bound) compared to apo-Tf (iron-depleted Tf) validates the potential of this approach in optimizing targeted delivery. Thus, FLIM-FRET can discriminate between TfR-Tf engagement and internalization into tumor cells from receptor-independent accumulation of Tf at the tumor region. This novel approach can be extended to other receptors, currently targeted in oncology. Hence, FLIM FRET can find numerous applications in drug delivery and targeted therapy assessment and optimization.

10069-45, Session 10

Frequency domain phosphorescence lifetime Imaging measurements and applications by ISS FastFLIM and multi pulse excitation

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Phosphorescence probes can have significantly long lifetimes, on the order of micro- to milli-seconds or longer. In addition, environmental changes can affect the lifetimes of these phosphorescence probes. Thus, Phosphorescence Lifetime Imaging Microscopy (PLIM) is a very useful tool to localize the phosphorescence probes based on their lifetimes to study the variance in the lifetimes due to the micro environmental changes. Since the probes respond to the biologically relevant parameters like oxygen concentration, they can be used to study various biologically relevant processes like cellular metabolism, protein interaction etc. In this case, we study the effects of oxygen on Oxyphor G4 with PLIM. Since The Oxyphor G4 can be quenched by O2, it is a good example of such a probe and has a lifetime around 250us. Here we present the digital frequency domain (PLIM) technique and study the lifetime of the Oxyphor G4 as a function of the O2 concentration. The lifetime data are successfully presented in a phasor plot for various O2 concentrations and are consistent with the time domain data. Overall, we can analyze the oxygen consumption of varying cells using this technique.

10069-46, Session 10

Rapid FLIM: The new and innovative method for ultra-fast imaging of biological processes

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Over the last two decades, time-resolved fluorescence microscopy has become an essential tool in Life Sciences thanks to measurement procedures such as Fluorescence Lifetime Imaging (FLIM), lifetime based Foerster Resonance Energy Transfer (FRET), and Fluorescence (Lifetime) Correlation Spectroscopy (F(L)CS) down to the single molecule level. Today, complete turn-key systems are available either as stand-alone units or as upgrades for confocal laser scanning microscopes (CLSM). Data acquisition on such systems is typically based on Time-Correlated Single Photon Counting (TCSPC) electronics along with picosecond pulsed diode lasers as excitation sources and highly sensitive, single photon counting detectors.

Up to now, TCSPC data acquisition is considered a somewhat slow process as a large number of photons per pixel is required for reliable data analysis, making it difficult to use FLIM for following fast FRET processes, such as signal transduction pathways in cells or fast moving sub-cellular structures. We present here a novel and elegant solution to tackle this challenge.

Our approach, named rapidFLIM, exploits recent hardware developments such as TCSPC modules with ultra short dead times and hybrid photomultiplier detector assemblies enabling significantly higher detection count rates. Thanks to these improved components, it is possible to achieve much better photon statistics in significantly shorter time spans while being able to perform FLIM imaging for fast processes in a qualitative manner and with high optical resolution. FLIM imaging can now be performed with up to several frames per second making it possible to study fast processes such as protein interactions involved in endosome trafficking.

10069-47, Session 10

Two-photon photoluminescence excitation and fluorescence lifetime spectroscopy for oil characterization

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Understanding the characteristics and chemical composition of petroleum is a challenge for researchers, due to large chemical composition and variety. It’s well-know that usual classification of oil is the API degree. In this work, the measurement of two-photon photoluminescence excitation spectroscopy (2p-PLE) and lifetime fluorescence spectroscopy was obtained to characterize and differentiate samples of Brazilian pre-salt petroleum, with different API degree, using a Ti:Sapphire with 100 fs pulses and 80 MHz repetition rate. The 2p-PLE and lifetime spectroscopy was acquired sweeping the laser excitation from 760 nm to 1000 nm in 5nm steps. For 2p-PLE we collected the emission from 350 nm to 650 nm, while for lifetime the collection was performed in the 380 nm to 680 nm range, using a spectral Becker&Hickl FLIM system. To improve the identification of different chemical composites of oil, we fractionated the oil in main components (asphaltenes, aromatics and polar). The results show successfully the spectral regions of chemical composites for each main component of petroleum and the differences between petroleum of different API degree. We anticipate that these techniques could be used satisfactorily for characterization and differentiation of different petroleum sources.

10069-48, Session 10

Fluorescence lifetime imaging of calcium flux in neurons in response to pulsed infrared light

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Pulsed infrared light can excite action potentials in neurons; yet, the fundamental mechanism underlying this phenomenon is unknown. Previous work has observed a rise in intracellular calcium concentration following infrared exposure, but the source of the calcium and mechanism of release is unknown. Here, we used fluorescence lifetime imaging of Oregon Green

BAPTA-1 to study intracellular calcium dynamics in primary rat hippocampal neurons in response to pulsed infrared light exposure. The fluorescence lifetime of Oregon Green BAPTA-1 is longer when bound to calcium, and allows robust measurement of intracellular free calcium concentrations. First, a fluorescence lifetime calcium calibration curve for Oregon Green BAPTA-1 was determined in solutions. The normalized amplitude of the short and long lifetimes was calibrated to calcium concentration. Then, neurons were incubated in Oregon Green BAPTA-1 and exposed to single pulses of infrared light (0-1 J/cm2; 0-5 ms; 1869 nm). Fluorescence lifetime images were acquired prior to, during, and after the infrared exposure. Fluorescence lifetime images were acquired for a duration of 10 ms and binned over repeated infrared exposures for sufficient signal. Results show the intracellular calcium flux following infrared exposure scales with irradiance. Furthermore, calcium dynamics and investigation with ion channel inhibitors suggests contributions from both outside and intracellular calcium stores. Altogether, this study characterizes calcium concentrations in neurons following pulsed infrared light exposure and elucidates sources and mechanisms of calcium transport.

10069-100, Session 10

A novel pulsed STED microscopy method using FastFLIM and the phasor plots

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Stimulated emission depletion (STED) microscopy is a powerful super-resolution microscopy technique; it allows observation of macromolecular complexes and sub-cellular structures with spatial resolution below the diffraction limit. The STED resolution is in theory unlimited, and typically increases upon the depletion laser power. However, increasing the depletion laser power could introduce several problems, such as photo-bleaching, photo-toxicity and anti-stoke emission background, etc. – these factors reduce the signal-to-noise ratio and thus lower the obtained resolution. More importantly, the photo-toxicity caused by the high depletion laser power can significantly limit STED applications to live specimens. Here, we present a novel STED microscopy method of using both pulsed excitation and pulsed depletion; it records the time-resolved photons by FastFLIM based on the digital frequency domain technique; in combination of the phasor plot approach, it can separate the completely depleted species from the partially depleted species based on their different decay kinetics, resulting in an increased resolution without increasing the depletion laser power. This new method also shows a promising multi-label STED application by using a single pair of excitation and depletion wavelengths – the fluorophores of different lifetimes are distinguished using the phasor plot approach.

10069-49, Session 11

From morphology to clinical pathophysiology: multiphoton fluorescence lifetime imaging at patients' bedside

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Application of multiphoton microscopy in the field of biomedical research and advanced diagnostics promises unique insights into the pathophysiology of skin diseases. By means of multiphoton excitation, endogenous biomolecules like NADH, collagen or elastin show autofluorescence or second harmonic generation. Thus, these molecules provide information about the subcellular morphology, epidermal architecture and physiological condition of the skin. To gain a deeper understanding of the linkage between cellular structure and physiological processes, non-invasive multiphoton-based intravital tomography (MPT) and fluorescence lifetime imaging (FLIM) were combined within the scopes of inflammatory skin, chronic wounds and drug delivery in clinical application.

The optical biopsies generated via MPT were morphologically analyzed and aligned with classical skin histology. Because of its subcellular resolution, MPT provided evidence of a redistribution of mitochondria in keratinocytes, indicating an altered cellular metabolism. Independent morphometric algorithms reliably showed a perinuclear accumulation in lesional skin in contrast to an even distribution in healthy skin. Confirmatively, MPT-FLIM showed an obvious metabolic shift in lesions. Moreover, detection of the onset and progression of inflammatory processes could be achieved. The feasibility of primary in vivo tracking of applied therapeutic agents further broadened our scope: We examined the permeation and subsequent distribution of agents directly visualized in patients´ skin in short-term repetitive measurements. Furthermore, we performed MPT-FLIM follow-up investigations in the long-term course of therapy.

Therefore, clinical MPT-FLIM application offers new insights into the pathophysiology and the individual therapeutic course of skin diseases, facilitating a better understanding of the processes of inflammation and wound healing.

10069-50, Session 11

Two-photon-excited fluorescence (TPEF) and fluorescence lifetime imaging (FLIM) with sub-nanosecond pulses and a high analog bandwidth signal detection

Matthias Eibl, Institut für Biomedizinische Optik, Univ. zu Lübeck (Germany); Sebastian N. Karpf, Univ. of California, Los Angeles (United States); Hubertus Hakert, Daniel Weng, Robert A. Huber, Institut für Biomedizinische Optik, Univ. zu Lübeck (Germany)

We present a two photon microscopy setup using a sub nanosecond pulsed multi-color fiber laser source synchronized to a high analog bandwidth signal detection for single shot two-photon-excited fluorescence (TPEF) imaging. The actively modulated pulses are adjustable from 50ps to 5ns at three different wavelengths -1064nm, 1122nm, and 1186nm- with kWs of peak power. At a repetition rate of 200kHz and 100ps pulse length, the duty cycle is comparable to typically used femtosecond pulses and thus the peak power is also comparable at same cw-power. Hence, both types of excitation should yield the same number of fluorescence photons per time on average when used for TPEF imaging. However, in the 100ps configuration, thousand times more fluorescence photons are generated per pulse.

In this presentation, we now show that the higher number of fluorescence photons per pulse combined with a high analog bandwidth detection makes it possible to not only use a single pulse per pixel for TPEF imaging but also to resolve the exponential time decay for fluorescence lifetime imaging (FLIM). To evaluate the performance of our system, we acquired multicolor images of labeled Cos7-Cells where each pixel of the image was excited by a single shot and compared them with ones acquired with a commercial ultra-short pulsed laser equipped two-photon microscope. Furthermore, we show FLIM images of a Convallaria sample (512x512 pixels, 1.8s total acquisition time) where the lifetime information is directly measured with a fast real

time digitizer. With the presented results, we show that longer pulses in the many-10ps to nanosecond regime can be readily applied for TPEF imaging and enable new imaging modalities like single pulse FLIM.

10069-51, Session 11

Monitoring conformational dynamics of the rotary subunit F in the A1-complex of archaeal ATP synthase by single-molecule FRET

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ATP synthases are large membrane-bound enzymes utilizing a proton or ion gradient and an electric membrane potential to catalyze the synthesis of ATP from ADP and phosphate. These ubiquitous enzymes also catalyze the reverse chemical reaction, i.e. the hydrolysis of ATP. To prevent any waste of ATP in the cell, several distinct control mechanisms exist for the ATP synthases in eubacteria, archaea, chloroplasts and mitochondria. It has been shown by single-molecule Forster resonance energy transfer (smFRET) experiments with FoF1-ATP synthase from the mesophilic bacterium Escherichia coli, that the C-terminus of the rotary subunit epsilon changes its conformation and inhibits ATP hydrolysis by mechanical blockade of the rotary movement of subunits. Here we investigate the function of the related subunit F of in the A1-complex of A1AO-ATP synthase from the archaea Methanosarcina mazei Gö1. Our smFRET data reveal nucleotide-dependent conformational changes of subunit F. However, these changes are in contrast to the regulatory movements found in the E. coli enzyme, and implicate a different regulatory mechanism of A1AO-ATP synthase.

10069-52, Session 11

Toward two-photon excited fluorescence lifetime endomicroscopy

Charles-Henri Hage, Pierre Leclerc, Marc Fabert, Julien Brevier, XLIM Institut de Recherche (France); Rémi Habert, Flavie Braud, Alexandre Kudlinski, Lab. de Physique des Lasers, Atomes et Molécules (France); Frédéric Louradour, XLIM Institut de Recherche (France)

Fluorescence lifetime imaging microscopy (FLIM) represents a powerful tool for biological studies. Endoscopic FLIM applied to the intracellular native biomarker NADH and FAD represents a promising mean for in vivo in situ malignant tissue diagnosis in the medical field. Else, 2-photon-excited fluorescence (2PEF) provides increased 3D resolution and imaging depth. But very few demonstrations about 2PEF lifetime measurement through a fiber have been reported and none about endoscopic 2P-FLIM through a practical fiber length (> 3m).

Our group has recently demonstrated the possibility to efficiently deliver through a very long optical fiber the short and intense excitation pulses required for 2P-FLIM. Our goal is now to check that collecting fluorescence through the same endoscopic fiber does not deteriorate the lifetime measurement. Relying on the basis previously published in case of 1PEF by P. French and co-workers (J. Biophotonics, 2015), we have experimentally quantitatively evaluated the influence on the lifetime measurement of the fiber chromatic and intermodal dispersions. The main result is that the fiber contribution to the system impulse response function, even in the case of a 3-meter long double-clad optical fiber, does not hinder the separation between free and bound NADH states using FLIM. Related calibrations and measurements will be detailed. Ongoing experiments about the development of a 2P-FLIM endomicroscope on the basis of an previously reported 2P-endomicroscope (Ducourthial et al., Sc. Reports, 2015), used under various configurations (i.e. point measurement in the center of the 2P-endomicroscope image, averaged lifetime, binned endoscopic 2P-FLIM image), will be also presented.

10069-53, Session 12

Correlated oxygen-sensing PLIM, cell metabolism FLIM and applications *(Invited Paper)*

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Cellular responses to oxygen tension have been studied extensively, optical imaging techniques based on time correlated single photon counting (TCSPC) to detect the underlying metabolic mechanisms are therefore of prominent interest. They offer the possibility by inspecting fluorescence decay characteristics of intrinsic coenzymes to directly image metabolic pathways. Moreover oxygen tension can be determined by considering the phosphorescence lifetime of a phosphorescent probe. The combination of both fluorescence lifetime imaging (FLIM) of coenzymes like NADH and FAD and phosphorescence lifetime (PLIM) of phosphorescent dyes could provide valuable information about correlation of metabolic pathways and oxygen tension.

A large variety of clinical phenotypes is associated with mitochondrial defects accomplished with changes in cell metabolism. In many cases the hypoxic microenvironment of cancer cells shifts metabolism from oxidative phosphorylation (OXPHOS) to anaerobic or aerobic glycolysis, a process known as “Warburg” effect. Also during stem cell differentiation a switch in cell metabolism is observed. A defective mitochondrial function associated with hypoxia has been invoked in many complex disorders such as type 2 diabetes, Alzheimers disease, cardiac ischemia/reperfusion injury, tissue inflammation and cancer. Within this work correlated imaging of phosphorescence and fluorescence lifetime parameters of metabolic markers were investigated and related to cell metabolism and oxygen tension of various diseases.

Publications:

[1] A. Rück, C. Hauser, S. Mosch, and S. Kalinina, “Spectrally resolved fluorescence lifetime imaging to investigate cell metabolism in malignant and nonmalignant oral mucosa cells”, Journal of Biomedical Optics 19(9), 096005 (2014).

[2] S. Kalinina, J. Breymayer, P. Schäfer, E. Calzia, V. Shcheslavskiy, W. Becker, and A. Rück, “Correlative NAD(P)H-FLIM and oxygen sensing-PLIM for metabolic mapping”, J. Biophotonics 1–12 (2016) / DOI 10.1002/jbio.201500297 (2016).

10069-54, Session 12

In vivo multiphoton imaging and quantification of cytoplasmic and nuclear metabolism in the hepatobiliary system

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In hepatobiliary metabolism, xenobioticis uptaken and processed in hepatocytes and later excreted into the bile canaliculi. In this work, we investigate theintracellular heterogeneity in such metabolic processes. 6-carboxyfluoresceindiacetate (6-CFDA) was used to investigate xenobiotic metabolism in hepatocytes with intravital multiphotonfluorescence microscopy. 6-CFDA is processed by intracellular esterase to fluorescent 6-CF, which can beimaged and quantified. We found that compared to the

nucleus, cytoplasmic 6-CF fluorescence intensityreached a maximum earlier following 6-CFDA injection. We also found a slight difference in the rate of 6-CFDA metabolism for cytoplasm and nucleus. These results suggest that molecular transport to the nucleus is additionally hindered and may affect drug transport there.

10069-55, Session 13

Genetically encoded sensors and fluorescence microscopy for anticancer research *(Invited Paper)*

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We report here on some results about the specific tumor cells parameters obtained by the fluorescence microscopy techniques (one-photon and two-photon microscopy, FLIM, STORM) and fluorescence whole-body imaging. A few parameters that potentially can change as a result of cancer transformation and anticancer treatment were studied – intracellular pH (pHi), hydrogen peroxide level, metabolic status, microviscosity, cytoskeleton, cell cycle, process of apoptosis. The study was performed on monolayer cell cultures, co-cultures of human cancer cells and fibroblasts, tumor spheroids and tumor xenografts. All parameters were investigated before and after anticancer treatment.

New genetically encoded ratiometric biosensors SypHer2 and HyPer2 based on the fluorescent protein cpYFP were used to detect pHi and hydrogen peroxide, correspondingly. Cell metabolism was analyzed by NADH and FAD fluorescence lifetime.

Also we analyzed metabolic changes, pHi and caspase-3 activation in cancer cells during apoptosis. The measurement of caspase-3 activation were performed using the genetically encoded FRET-based sensor for caspase-3 activity mKate2-DEVD-iRFP.

Cell cycle phases in viable cells were visualized using a new FLIM-FUCCI sensor based on two fluorescent proteins with different fluorescence lifetime (mCherry-hCdt1 and mKate2-Geminin).

Red fluorescent protein capable of intrinsic blinking was applied to observe ultrafine changes of cancer cell cytoskeleton, such as increase in size of focal contact sites or point-like actin structures less than diffraction barrier. Also we proposed a protocol of super-resolution fluorescence imaging with recently reported SiR-actin dye allowing us to reveal a complex network of thick curved actin bundles in cancer cells in tumor tissue.

Using fluorescent Bodipy-based molecular rotors and FLIM cellular microviscosity was estimated in cancer cells. We showed that microviscosity in cancer cells the plasma membrane is more viscous than in normal cells.

Finally, we can conclude, that all parameters are dramatically changed during cancer transformation and in the process of anticancer treatment.

10069-56, Session 13

Effects of anti-cancer drug doxorubicin on endogenous biomarkers NAD(P)H, FAD & Trp in prostate cancer cells: a FLIM Study *(Invited Paper)*

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Fluorescence Lifetime Imaging (FLIM) can be used to identify metabolic changes during cancer progression and upon anti-cancer drug treatment. Prostate cancer (PCa) is one of the leading cancers in men in the USA. Mitochondrial dysfunction and defective OXPHOS (oxidative phosphorylation) activity has been reported in cancer. The anti-cancer drug doxorubicin has been shown to induce apoptosis. Induction of cell death by correction of mitochondrial activity is a promising strategy of cancer treatment. This research focusses on understanding the changes in NAD(P)H, FAD and Trp in mitochondria of LNCaP PCa cells upon treatment with doxorubicin using our 3-channel FLIM-FRET approach and by quantitative analysis of cellular metabolism. Live cell FLIM-FRET measurements on LNCaP cells were done on Zeiss 780 2p confocal microscope coupled with B&H TCSPC FLIM board. The same FoV (fields of view) were imaged both before treatment and in 15 minute intervals after adding 0.5 μM doxorubicin up to 60 min when the cells started to die. Increase in NAD(P)H ?m, increase in NAD(P)H enzyme bound fraction, decrease in FAD enzyme bound and reduction in the NAD(P)H/FAD redox ratio was seen upon doxorubicin treatment. Increase in Trp-E% was also seen upon doxorubicin treatment which corresponds to the increase in the NAD(P)H enzyme bound fraction. Our results are very encouraging and demonstrate that FLIM-FRET has the capability to detect early changes associated with the correction in mitochondrial/OXPHOS activity and induction of apoptosis upon treatment with doxorubicin in PCa cells.

10069-57, Session 13

Fluorescence lifetime imaging of endogenous molecules in live mouse cancer models

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NADH and FAD are important endogenous fluorescent coenzymes participating in key enzymatic reactions of cellular metabolism. While fluorescence intensities of NADH and FAD have been used to determine the redox state of cells and tissues, this simple approach breaks down in the case of deep-tissue intravital imaging due to depth- and wavelength-dependent light absorption and scattering. To circumvent this limitation, our research focuses on fluorescence lifetimes of two-photon excited NADH and FAD emission to study the metabolic state of live tissues. In our custom-built scanning microscope we combine tunable femtosecond Ti:sapphire laser (operating at 740 nm for NADH excitation and 890 nm for FAD excitation), two GaAsP hybrid detectors for registering individual fluorescence photons and two Becker and Hickl time correlator boards for high precision lifetime measurements. Together with our rigorous FLIM analysis approach (including image segmentation, multi-exponential decay fitting and detailed statistical analysis) we are able to detect metabolic changes in cancer xenografts (human pancreatic cancer MPanc96 cells injected subcutaneously into the ear of an immunodeficient nude mouse), relative to surrounding healthy tissue. Advantageously, with the same instrumentation we can also take high-resolution and high-contrast images of second harmonic signal (SHG) originating from collagen fibers of both the healthy skin and the growing tumor. The combination of metabolic measurements (NADH and FAD lifetime) and morphological information

(collagen SHG) allows us to follow the tumor growth in live mouse model and the changes in tumor microenvironment.

10069-58, Session 13

Quantitative metabolic microendoscopy within a living organism based on two-photon excited endogenous molecular imaging of intracellular NADH and FAD

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Multiphoton microscopy is a cutting edge imaging modality leading to increasing advances in biology and also in the clinical field. To use it at its full potential and at the very heart of clinical practice, there have been several developments of fiber-based multiphoton microendoscopes. The application for those probes is now limited by few major restrictions, such as the difficulty to collect autofluorescence signals from tissues and cells theses being inherently weak (e.g. the ones from intracellular NADH or FAD metabolites). This limitation reduces the usefulness of microendoscopy in general, effectively restraining it to morphological imaging modality requiring staining of the tissues. Our aim is to go beyond this limitation, showing for the first time label-free cellular metabolism monitoring, in vivo in situ in real time.

The experimental setup is an upgrade of a recently published one (Ducourthial et.al, Scientific Reports, 2016) where femtosecond pulse fiber delivery is further optimized thank's to a new transmissive-GRISM-based pulse stretcher permitting high energy throughput and wide bandwidth. This device allows fast sequential operation with two different excitation wavelengths for efficient two-photon excited NADH and FAD autofluorescence endoscopic detection (i.e. 860 nm for FAD and 760 nm for NADH), enabling cellular optical redox ratio quantification at 8 frames/s.

The obtained results on cell models in vitro and also on animal models in vivo (e.g. neurons of a living mouse) prove that we accurately assess the level of NADH and FAD at subcellular resolution through a 3-meters-long fiber with our miniaturized probe (O.D. =2.2 mm).

10069-59, Session 13

A new and sensitive method of analyzing metabolic activity using fluorescence lifetime imaging microscopy of NADH

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Traditional assessments of cellular metabolism are often destructive, time consuming and without visual information. Fluorescence lifetime imaging microscopy (FLIM) provides a highly sensitive, non-invasive, and label-free alternative.

This study uses FLIM in combination with two-photon microscopy to investigate pharmacological induced metabolic changes of adipocytes via changes in the fluorescence of the metabolic co-factors NADH and FAD. In agreement with recent publications NADH fluorescence suggests the presence of four distinct lifetimes in cell culture and tissue with two unbound and two protein bound states which show different responses to treatment with metabolic modifiers. We evaluated the effects on NADH fluorescence lifetime after systematic manipulations to change the balance between oxidative and glycolytic metabolism using five pharmacological reagents - Oligomycin, 2-DG, FCCP, Rotenone, and Glucose - which interact with different parts of the metabolic pathway. We established several ratios between the four distinct lifetimes of NADH after treatment and compared the results to oxygen consumption rate and extracellular acidification rate.

We demonstrated, for the first time, a correlation between the two unbound fluorescence lifetimes components and glycolytic and oxidative metabolic activity with a significant higher sensitivity compared to the commonly used free-to-bound ratio of NADH. Analyzing all four lifetime components of NADH has the potential to become a powerful tool to evaluate metabolic activity of adipocytes with subcellular resolution.

10069-60, Session 13

Pump-probe microscopy of respiratory chain pigments: towards non-fluorescent label-free metabolic imaging

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Current label-free metabolic microscopy techniques are limited to obtaining contrast from fluorescent molecules NAD(P)H and FAD+, and are unable to determine redox state along the mitochondrial respiratory chain itself. Several non-fluorescent respiratory chain electron carriers are heme proteins that have redox-dependent absorption spectra. The most prominent of these, cytochrome c, has been extensively characterized by transient absorption spectroscopy, which suggests that a pump-probe measurement with pulses in the range of 450 – 600 nm can provide strong contrast between its redox states. Motivated by the success of pump-probe microscopy targeting another heme protein, hemoglobin, we seek to extend the technique to the cytochromes, with the ultimate goal of dissecting respiratory chain function of individual cells in live tissue.

To that end, we have developed a new optical system producing ultrafast, visible, independently-tunable

pulse pairs via sum-frequency generation of nonlinearly broadened pulses in periodically-poled lithium niobate. The system is pumped by a homebuilt fiber-based oscillator/amplifier emitting 1060 nm pulses at 1.3 W (63 MHz repetition rate), and produces tunable pulses in the vicinity of 488 and 532 nm. Pump-probe spectroscopy of cytochrome c with this source reveals differences in excited-state absorption relaxation times between redox states. Moreover, pump-probe images were acquired of brown adipose tissue (which contains dense mitochondria), and also heart muscle. Differences were observed in the averaged time-dependent pump-probe response between the two tissues, presumably due to the myoglobin present in muscle, a very encouraging initial result for this promising tool for heme specific imaging using visible pump-probe imaging.

10069-61, Session 14

Absorption characterization of immersion medium for multiphoton microscopy at the 1700-nm window

Ping Qiu, Wenhui Wen, Ke Wang, Shenzhen Univ. (China)

The 1700-nm window has emerged as a promising excitation window for multiphoton microscopy (MPM). On one hand, the combined low tissue absorption and scattering make this window well suited for deep-tissue MPM; on the other hand, the long excitation wavelength makes higher-order MPM in biological tissues feasible, e.g., recently 4-photon fluorescence MPM in mouse brain has been demonstrated. Objective lens is a key optical component in the entire MPM setup. Multiphoton signal levels are largely dependent on the transmittance of objective lens. Here we demonstrate experimental results of transmittance measurement of two water immersion objective lenses commonly used for MPM at the 1700-nm window, covering both the excitation and the signal window. Our target application is MPM of even higher order excited at this window, i.e., 4th harmonic generation (FHG) imaging and 5-photon fluorescence imaging. Our results show that, although the customized objective lens offers higher transmission at the excitation window, it suffers from dramatically degraded transmittance at the signal window, compared with the non-customized objective lens. These results will offer guidelines for selection of proper objective lens for higher-order MPM at the 1700-nm window.

10069-62, Session 14

Identification of intramural metastasis in esophageal cancer using multiphoton microscopy

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Intramural metastasis (IM) of esophageal cancer is defined as metastasis from a primary lesion to the esophageal wall without intraepithelial cancer extension. Esophageal cancer with IM is more common and such cases indicate a poor prognosis. In esophageal surgery, if curative resection is possible, the complete removal of both primary tumor and associated IMs is required. Therefore, accurate diagnosis of IMs in esophageal cancer prior to surgery is of particular importance. Multiphoton microscopy (MPM) with subcellular resolution is well-suited for deep tissue imaging since many endogenous fluorophores of fresh biological tissues are excited through two-photon excited fluorescence (TPEF) and second harmonic generation (SHG). Here, a pilot study to diagnose IM in fresh tissue section using MPM is reported. Morphological features of IM were first shown. Then, the morphological and spectral differences between intramural metastic lesion and the main components of submucosa and muscularis propria were described. Quantitative parameters including the nuclear-to-cytoplasmic ratio of cells and the pixel density of collagen were also extracted. These results show that MPM has the ability to accurately diagnose IMs in esophageal cancer. With improvement of the penetration depth of MPM and the development of multiphton microendoscope, MPM may be a promising imaging technique for preoperative diagnosis of IMs in esophageal cancer in the future.

10069-63, Session 14

Ten years of biophotonics single-photon SPAD imager applications: retrospective and outlook

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Individual SPADs (single-photon avalanche diodes), capable of detecting and time-stamping single optical photons, have long been known and appreciated for their excellent timing resolution. The breakthrough implementation of the first SPADs in standard CMOS technologies (2003) has opened the possibility of low-cost volume fabrication of digital SPAD imagers, potentially providing unparalleled time-resolved sensing performance. A host of architectures and target applications have been explored since. The former range from simple pixel arrays, with off-chip data processing electronics, possibly on FPGAs to implement modular set-ups ("reconfigurable pixels"), to fully integrated "smart" imagers with in-pixel time-stamping electronics and/or on-chip data processing fabric. Biomedical applications include (endoscopic) FLIM, (multi-beam multiphoton) FLIM-FRET, SPIM-FCS, time-resolved Raman, NIROT, localization- and entangled photons-based super-resolution microscopy, and PET (Positron Emission Tomography), to name a few. SPAD arrays do have some weak points as well, such as their quantum efficiency and fill factor, which somehow still lag behind those of CCDs and sCMOS imagers, as well as their pixel sizes, which are typically larger, thereby limiting at present practical overall imager sizes. Data handling and sensor operation have also to be properly addressed, which often leads to important firmware development efforts. On the other hand, this opens the door to real-time algorithmic implementations close to the sensor itself, such as FPGA-based autocorrelation and lifetime calculation.

We will review some representative sensors and applications, the corresponding challenges, in particular compared to established devices as well as alternative CMOS imagers, and provide an outlook on the future of this fascinating technology.

10069-64, Session 14

High-speed amplitude modulation of femtosecond laser pulses for frequency-multiplexed two photon imaging

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Two-photon laser scanning microscopy (TPLSM) has become a widely used tool for imaging neuron morphology and function in intact cortex. TPLSM typically provides data acquisition rates of about 10 Mpixels/s and allows for observation of fluorescently labeled neurons within limited regions-of-interest at 5 – 30 frames/s. A complimentary approach for studying function of distributed sparsely labeled neurons in a volume termed "random-access scanning" supports switching between user-selected recording sites in 2D or even 3D with a positioning time of 10 – 20 μs and achieves scan rates of 30 -50 Kpixels/s, allowing interrogation at ~500 sites with 10 ms temporal resolution. Further improvements of TPLSM and random access scanning are possible with multiplexing approaches which enable simultaneous multi-site imaging with multiple excitation beams and a single detector.

In this work we present a novel frequency-multiplexed two photon imaging method that utilizes high-speed amplitude modulation of fs laser pulses in the MHz range to tag each excitation beam and the corresponding fluorescence signals with specific beat frequencies. Frequency tags are generated with an interferometric setup including acousto-optic deflectors (AODs) to achieve precise spatial overlap of femtosecond pulses with acoustically-shifted frequencies. By creating the matching excitation beam patterns in each interferometer arm using multiple AOD driving frequencies, and subsequently overlapping these matching patterns results in multiple excitation beams with unique amplitude-modulated beat frequencies available for scanning. Demultiplexing of the composite fluorescence signal is done by standard lock-in detection procedures. Proof-of-concept work, physics of the effect and preliminary imaging results will be discussed.

10069-65, Session 14

Spectrally-encoded multi-photon microscopy

Sebastian Nino Karpf, Bahram Jalali, Univ. of California, Los Angeles (United States)

A new tool for kHz frame-rate multi-photon microscopy is presented. We harness the broadband light of a rapidly wavelength-swept FDML laser, which is modulated and amplified for high peak powers, to achieve line-scanning rates of 342kHz, an order of magnitude faster than resonant galvanometric mirrors. The inertia limited speed of galvanometric mirrors is overcome by employing passive, diffraction-based scanning. We developed a wavelength-swept FDML laser which is actively modulated to 100ps pulses by an electro-optical modulator (EOM) at up to 88MHz repetition rate and amplified by ytterbium-doped fiber amplifiers (YDFAs). By sending these spectrally swept pulses on a diffraction grating, a spectral brush is generated. Thus, pixels along a line are illuminated sequentially in time, with the repetition rate corresponding to the pixel-rate at up to 88MHz (programmable). The line-rate is given by the sweep-rate of the FDML (342kHz) and can go beyond MHz rates. The y-axis is scanned with a galvanometric mirror. We show image rates surpassing 1000 frames per second. A single photomultiplier tube (PMT) is employed for detection. A further contrast is established by extracting the fluorescence lifetime images of the same data, generating parallel fluorescence lifetime images (FLIM) at kHz frame-rates. This system, coined Two-Photon Imaging by diffracted swept-laser excitation (TIDE), is already fiber-based, without spurious non-linear interaction in the fiber due to the 100ps pulses and thus a promising new tool for multi-modal, non-linear endoscopic imaging.

10069-66, Session 14

Dynamical measurements of motion behavior of free fluorescent sphere using the wide field temporal focusing microscopy with astigmatism method

Chi-Hsiang Lien, National United Univ. (Taiwan); Chun-Yu Lin, National Cheng Kung Univ. (Taiwan); Shean-Jen Chen, Institute of Imaging and Biomedical Photonics, National Chiao Tung Univ. (Taiwan); Fan-Ching Chien, National Central Univ. (Taiwan)

A three-dimensional (3D) single fluorescent particle tracking strategy based on temporal focusing multiphoton excitation microscopy (TFMPEM) combined with astigmatism imaging is proposed for delivering nanoscale-level axial information that reveals 3D trajectories of single fluorospheres in the axially-resolved multiphoton excitation volume without z-axis scanning. It provides the dynamical ability by measuring the diffusion coefficient of fluorospheres in glycerol solutions with a position standard deviation of 14 nm and 21 nm in the lateral and axial direction and a frame rate of 100 Hz. Moreover, the optical trapping force based on the TFMPEM is minimized to avoid the interference in the tracing measurements compared to that in the spatial focusing MPE approaches. Therefore, we presented a three dimensional single particle tracking strategy to overcome the limitation of the time resolution of the multiphoton imaging using fast frame rate of TFMPEM, and provide three dimensional locations of multiple particles using an astigmatism method.

10069-67, Session 14

Label-free carbon particulates detection using femtosecond pulsed illumination

Christian Steuwe, KU Leuven (Belgium); Hannelore Bové, Univ. Hasselt (Belgium) and KU Leuven (Belgium); Martin J. vandeVen, Marcel Ameloot, Univ. Hasselt (Belgium); Maarten B. J. Roeffaers, KU Leuven (Belgium)

The adverse health effects of particulate matter exposure are a generally accepted concern. Dramatic statistical figures suggest that fine dust is a main environmental risk in Europe and can be held accountable for hundreds of thousands of deaths per year [1]. Locating and tracking these nanometer sized particles, however, is not straight forward: In epidemiological and toxicology research only measurements based on labels [2] such as radionuclide markers have been applied.

In this paper we present a direct, label-free optical contrast mechanism to detect carbon nanoparticles immersed in aqueous environments [3]. The virtue of this technique is its ability to perform in body fluids such as urine but also in cells and tissues. The mechanism is based on white light (WL) generation upon illumination with femtosecond pulsed near-infrared and is therefore non-incandescence related. We demonstrate the technique in various biological settings with dry and suspended carbon black particles (CB), a widely used model compound for soot [4]. Our approach allows for the unequivocal localization of CB alongside of common fluorophores and markers and can be performed on multiphoton laser-scanning microscopy platforms, a system commonly available in research laboratories.

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10070-1, Session 1

Multi-objective tomographic microscope using LED array illumination

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We demonstrate a two-objective tomographic microscope with a coded source for capturing data and reconstructing 3D refractive index distributions of semitransparent samples. Our approach uses a programmable LED array to pattern illumination angles, combined with two objective lenses, tilted with respect to each other, and a single sensor. We show that this setup is able to record projections of the sample from different viewpoints covering a large volume of 3D Fourier space. Our prototype combines two configurations that are used in optical tomography: sample rotation and illumination rotation. Illumination rotation for tomography provides improved lateral resolution and can be done with no moving parts; however, the axial resolution is limited and overall resolution is highly anisotropic in 3D. Sample rotation can provide uniform coverage of 3D spatial frequencies and more isotropic resolution, but suffers from inaccurate and difficult sample rotation mechanisms, particularly for microscopic single cell samples. Our method combines the best of both approaches, having no moving parts and avoiding complicated sample rotation but still achieving extended coverage in frequency space compared to a single objective approach. We further use intensity-only measurements to combine phase retrieval and 3D reconstruction into a joint algorithm. This eliminates the need for interferometric reference beams that are typically used in optical diffraction tomography. We compare linear inversion algorithms that use a single-scattering approximation to non-linear methods, which can handle multiple scattering.

10070-2, Session 1

Region of interest and sub-volume optical projection tomography for high resolution 3D imaging of specific volumes within in vivo specimens on a commercial widefield microscope

Thomas J. Watson, Natalie Andrews, Laurence Bugeon, Margaret J. Dallman, Paul M. W. French, James McGinty, Imperial College London (United Kingdom)

Optical Projection Tomography (OPT) is a technique that can measure the absorption and/or fluorescence distribution in 3D samples including -transparent model organisms in vivo such as C.elegans and D.rerio (zebrafish). Our simple design adapts existing widefield commercial microscopes for OPT, increasing the functionality to include anatomical and functional 3D imaging.

The conventional approach to OPT requires the depth of field (DoF) of the imaging system to cover the front half of the sample, limiting the numerical aperture, light collection and spatial resolution. This trade-off can be overcome by implementing focal scanning such that high resolution information is acquired from all depths. We will describe two opportunities presented by focal scanning OPT, namely region-of-interest (RoI-) OPT and sub-volume (SV-) OPT.

RoI-OPT restricts the extent of axial focal scanning to increase the signal-to-noise for a specified region within the sample by suppressing the contribution outside through defocus. This simple implementation is restricted to low magnification (4-10), such that the whole specimen remains within the field of view.

SV-OPT is the natural extension of RoI-OPT, increasing the magnification (20-50), using compound focal scanning and more complex reconstruction geometry. The control of the DoF and scan range is used to circumvent the ill-posed problem presented by image truncation in pure projection.

Both techniques can image small volumes within larger samples, such as specific organs inside live zebrafish, with the simpler implementation RoI-OPT or SV-OPT for high magnification imaging. We will present these novel focal scanning OPT techniques for in vivo imaging of zebrafish embryos.

10070-3, Session 1

Implementation of an incoherent 3D patterned illumination design in a structured illumination microscope

Sharon V. King, Christopher Taylor, Ana Doblas, Hasti Shabani, Nurmohammed Patwary, The Univ. of Memphis (United States); Genaro Saavedra, Univ. de València (Spain); Chrysanthe Preza, The Univ. of Memphis (United States)

This paper investigates experimental adaptation of an incoherent 3D patterned illumination system [1] to application in a prototype SIM imaging system. The illumination system, based on an incoherent source, a set of parallel slits and a beam-splitting Fresnel bi-prism, generates localized interference fringes with a wide, continuously tunable range of lateral and axial spatial frequencies that are not easily accessible using other existing methods of generating structured illumination [2]. We present experimental data that validates the functionality of the illumination system in a wide-field fluorescence microscope. Numerical simulations and an experimental SIM system are used to compare theoretical and practical system properties. Results confirm the relationships between design parameters and system properties that contribute to the flexibility of the system and its operation in both the optical sectioning regime [3, 4] and the super-resolution regime [2]. These results also determine the accuracy of simulation for use as a design tool. Specifically, by moving the axial position of the beam-splitting prism, the lateral and axial modulation frequencies of the illumination may be varied within a range determined by the distribution of the slits and the properties of the prism. As part of an imaging system, this illumination approach can enable a SIM with adjustable properties while affording the advantages of wide-field incoherent microscopy in terms of reduced cost and reduced light intensity.

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10070-4, Session 1

Toward multi-focal spot remote focusing two-photon microscopy for high speed imaging

Bei Li, Alexander D. Corbett, Ee Zhuan Chong, Edward Mann, Tony Wilson, Martin J. Booth, Gil Bub, Univ. of Oxford (United Kingdom)

Scanning two-photon microscopy is a powerful tool used for in-depth, high resolution three-dimensional (3D) sectioning. In order to observe the dynamic behaviour of biological specimens, high speed 3D image generation is required. Traditionally, 3D imaging data is collected by physically changing the distance between the objective lens and specimen. This is problematic for two reasons. First, this process is generally slow and second it can lead to undesirable specimen agitation. Remote focussing is a technique which enables optically sectioned, in-focus images of a specimen to be taken outside of the focal plane. For this reason, it's an appropriate solution to achieve high speed 3D imaging without any agitation of the specimen. We have developed a fast remote focussing system which uses a reference objective to axially displace the focal spot at 500Hz rates. This is a significant development as it allows images of the entire specimen volume to be acquired without the need to move either the specimen or the objective. Moreover, the ability to scan over large depth ranges can be achieved at speeds equivalent to lateral scan speeds, allowing fast events in excitable cell networks to be captured. In addition, we have also combined temporal pixel multiplexing (TMP) and remote focussing for simultaneous high-speed, high-resolution imaging. This new imaging platform will be used for recording cellular activity and high spatial resolution imaging of anatomical structure.

10070-5, Session 1

Volumetric retinal microscopic imaging with greatly extended depth of field by home-made general cubic phase mask

Zengzhuo Li, The Ohio State Univ. (United States); Andrew Fischer, The Ohio State Univ. (United States); Wei Li, National Eye Institute (United States); Guoqiang Li, The Ohio State Univ. (United States)

Wavefront-engineering techniques have been demonstrated to be of great value in extending depth of field (EDoF). Bright-field infinity-corrected transmission/reflection microscopes have been built with Kohler illumination. By employing our home-made and custom designed general cubic phase mask, the DoF of the microscope systems is greatly improved. The phase mask is placed in between objective lens and tube lens and designed using the optical design software (Zemax). The resulting EDoF system is optimized to achieve a point spread function (PSF) that is less sensitive to defocus than the conventional microscope system so that the DoF could be significantly improved. In Zemax simulation for a system using 32X/0.6NA objective lens, this method could extend the DoF about 13 times. And in another example of both simulation and experiment, a 20X/0.4NA objective lens was used and the corresponding general cubic phase mask was designed and fabricated. The DoF has been extended by more than 12 times in the experiment. With this new approach, both the resolution and contrast have been improved at the focal plane and at other positions out of focus within a 40 μm depth range. The resolution is kept the same as the diffraction-limited value in the EDoF. This method has been demonstrated to be promising in 3D microscope imaging.

10070-6, Session 2

Multimodal microscopy for extraction of refractive indices in live cells

Natan T. Shaked, Tel Aviv Univ. (Israel)

We present our latest advances in combining label-based microscopy and label-free interferometric microscopy for extraction of the refractive index of live cells growing in three dimensions. Label-based microscopy is used to enable molecular specificity, providing the ability to selectively see organelles of interest inside the cells. Label-free interferometric microscopy, on the other hand, provides the ability to quantitatively visualize the optical thickness maps of the cells. Combining both modalities yields a new platform of learning on the refractive indexes of certain cell organelles, which, as going to be shown using various experimental demonstrations, has a high potential for clinical use.

10070-7, Session 2

Lensfree diffractive tomography for the imaging of 3D cell cultures

Anthony Berdeu, CEA Grenoble (France); Fabien Momey, Lab. Hubert Curien (France); Jean-Marc Dinten, MINATEC (France); Xavier Gidrol, Nathalie Picollet-D'hahan, CEA Grenoble (France); Cédric Allier, CEA-LETI (France)

New microscopes are needed to help realize the full potential of 3D organoid culture studies by gathering large quantitative and systematic data over extended period of time while preserving the integrity of the living sample.

In order to reconstruct large volume while preserving the ability to catch every single cell, we propose new imaging platforms based on lensfree microscopy, a technic which is addressing these needs in the context of 2D cell culture, providing label-free and non-phototoxic acquisition of large datasets. We have built lensfree diffractive tomography setups performing multi-angle acquisitions of 3D organoid culture embedded in Matrigel® and developed dedicated 3D holographic reconstruction algorithms based on the Fourier diffraction theorem.

Nonetheless, holographic setups do not record the phase of the incident wave front and the biological samples in Petri dish strongly limit the angular coverage. These limitations introduces numerous artefacts in the sample reconstruction. We developed several methods to overcome them, such as multi wavelength imaging or iterative phase retrieval. The most promising technic currently developed is based on a regularized inverse problem approach directly performed on the 3D volume to reconstruct.

3D reconstructions were realized on several complex samples such as 3D networks or spheroids embedded in capsules with large reconstructed volumes up to ~25 ????3 while still being able to identify single cells.

To our knowledge, this is the first time that such an inverse problem approach is implemented in the context of lensfree diffractive tomography enabling to reconstruct large volume of labefree biological samples.

10070-8, Session 2

Focus-tuneable lens in limited-angle holographic tomography

Arkadiusz T. Ku?, Wojciech Krauze, Malgorzata Kujawi?ska, Warsaw Univ. of Technology (Poland)

Limited-angle, holographic tomography (HT) is a technique, in which a set of projections (holograms) of a stationary sample is acquired at different viewing directions and then, using Fourier diffraction theorem, the three-dimensional refractive index distribution is reconstructed. This quantitative information allows imaging and measuring transparent objects, thus

analyzing for example the 3D morphology of living cancer cells, which is one of the hot topics in biology. However, high numerical aperture of microscope objectives used here results in shallow depth of field of the acquired projections and spoils the reconstruction quality away from the object plane. While it is possible to numerically propagate each projection to extend the depth of field, it is usually a time-consuming and computationally demanding operation. What is more, propagating highly off-axis fields is usually inaccurate. In this paper we demonstrate a holographic tomography system using an electrically-tuneable lens instead of numerical propagation. The lens is placed in a common-scan, Mach-Zehnder-based system having the architecture optimized in order to incorporate this new component. The imaging part of the optical system is stationary, which means that for refocusing purposes the z-stage is used. Apart from providing hardware propagation, the tuneable lens also acts as a precise z-stage substitution. We also propose optimum number of refocusing planes in order to achieve the best compromise between measurement time increase and accuracy improvement. In this work the benefits of using the hardware refocusing are verified with an example of mouse myoblast cells studies.

10070-9, Session 2

Simple and fast spectral domain algorithm for quantitative phase imaging of living cells with digital holographic microscopy

Junwei Min, Westfälische Wilhelms-Univ. Münster (Germany) and Xi'an Institute of Optics and Precision Mechanics (China); Baoli Yao, Xi'an Institute of Optics and Precision Mechanics, CAS (China); Steffi Ketelhut, Björn Kemper, Westfälische Wilhelms-Univ. Münster (Germany)

Digital Holographic Microscopy (DHM) has been proven to be a powerful tool for quantitative live cell imaging. The combination of DHM with conventional optical microscopes simplifies the usage of the technique as microscope objectives (MO) can be easily changed and the condenser allows simple adjustment of the illumination. However, the high flexibility of illumination and imaging also causes variable phase aberrations that need to be considered during the hologram reconstruction process. Many works on the successful compensation of such phase aberrations have already been reported but appear often unpractical for variable illumination and imaging systems. For example, the introduction of the same phase aberration in the reference arm leads to more complex and expensive systems and also requires a precise adjustment of the optical elements. The recording of additional specimen-free reference holograms cannot be adapted to changes of the optical system, e.g. during long-term experiments. In practice, phase aberrations were also successfully eliminated during the digital reconstruction process by using numerical fitting methods. However, those methods need pre-knowledge about setup and sample or high computational requirements which can prevent on-line monitoring. We propose a novel numerical method for phase aberration compensation that is based on the analysis of the hologram's 2D-frequency spectrum that it capable for on-line quantitative phase imaging. From a single shot off-axis hologram, the whole phase aberration can be eliminated automatically without fitting or pre-knowledge of the setup. After characterization of the method by simulations, the capabilities for quantitative phase imaging of living cancer cells are demonstrated.

10070-10, Session 3

Probing neural tissue with Airy light-sheet microscopy: investigation of imaging performance at depth within turbid media

Jonathan Nylk, Kaley McCluskey, Sanya Aggarwal, Javier A. Tello, Kishan Dholakia, Univ. of St. Andrews (United Kingdom)

Light-sheet microscopy (LSM) has received great interest for fluorescent imaging applications in biomedicine as it facilitates three-dimensional visualisation of large sample volumes with high spatiotemporal resolution whilst minimising irradiation of, and photo-damage to the specimen. Despite these advantages, LSM can only visualise superficial layers of turbid tissues, such as mammalian neural tissue.

Propagation-invariant light modes have played a key role in the development of high-resolution LSM techniques as they overcome the natural divergence of a Gaussian beam, enabling uniform and thin light-sheets over large distances. Most notably, Bessel and Airy beam-based light-sheet imaging modalities have been demonstrated. In the single-photon excitation regime and in lightly scattering specimens, Airy-LSM has given competitive performance with advanced Bessel-LSM techniques.

Airy and Bessel beams share the property of self-healing, the ability of the beam to regenerate its transverse beam profile after propagation around an obstacle. Bessel-LSM techniques have been shown to increase the penetration-depth of the illumination into turbid specimens but this effect has been understudied in biologically relevant tissues, particularly for Airy beams. It is expected that Airy-LSM will give a similar enhancement over Gaussian-LSM.

In this paper, we report on the comparison of Airy-LSM and Gaussian-LSM imaging modalities within cleared and non-cleared mouse brain tissue. In particular, we examine image quality versus tissue depth by quantitative spatial Fourier analysis of neural structures in virally transduced fluorescent tissue sections, showing a three-fold enhancement at 50?m depth into non-cleared tissue with Airy-LSM. Complimentary analysis is performed by resolution measurements in bead-injected tissue sections.

10070-12, Session 3

Fractal propagation method enables realistic and rapid simulations of light propagation for optical-sectioning microscopy

Adam K. Glaser, Ye Chen, Jonathan T. C. Liu, Univ. of Washington (United States)

Although many simulation methods exist for modeling light transport in biological media (e.g., the diffusion approximation, Monte Carlo, and finite-difference time-domain), each has limitations for the efficient and realistic modeling of three-dimensional microscopic light transport in biological tissues with refractive heterogeneities. To address this need, we describe a novel technique, the fractal propagation method (FPM), which incorporates the beam propagation method - valid for modeling light transport in media with weak variations in refractive index - along with a fractal model of refractive index turbulence. In contrast to standard simulation methods, the FPM is able to accurately and efficiently simulate the diffraction effects of focused beams, as well as the microscopic heterogeneities present in tissue that result in scattering, refractive beam steering, and the aberration of beam foci. We compare the developed method to theoretically derived expressions for light scattering in a fractal medium in order to validate the technique and the relationship between the FPM model parameters (the fractal dimension, correlation length, mean refractive index, and variance of refractive index fluctuations) and the more conventional optical parameter used to describe tissues (the scattering coefficient). We demonstrate the flexibility and robustness of the technique by examining the steering and distortion of Gaussian and Bessel beams in tissue with comparison to experimental data. Finally, we demonstrate the application of the FPM method for modeling the illumination and collection optics of a light-sheet microscope.

10070-13, Session 3

An iterative algorithm for designing XZ intensity distribution

Tong Ye, Clemson Univ. (United States)

Bessel beam belongs to the typical class of non-diffractive optical fields with an extremely long focal profile. Bessel beams are widely used both in the linear and nonlinear regime such as light sheet microscopy, optical coherence tomography, optical trapping. However, ideal Bessel beams only rigorously exist in theory; the generated Bessel beams in the lab are quasi-Bessel beams with finite propagation regions and varying intensity profiles along propagation axis, which may affects imaging applications that need uniform illumination.

In this report, we propose an iterative Fourier-transform algorithm for creating holograms that can diffract light into an arbitrary two-dimensional intensity profile in XZ plane, where the Z denotes the propagation direction. This algorithm is computationally efficient by fast Fourier-transform, and finishes the calculation in a few seconds. In experiments, a spatial light modulator (SLM), being addressed with the calculated holograms, is illuminated by a laser beam and is relayed to the back focal plane of a focusing objective. The desired beam profiles are produced in the XZ plane which extends along optical axis and is perpendicular to the focal plane of the objective. Using this method, the on-axis intensity profile can be designed and tuned according to the applications. For example, a specific designed focal field can be generated and scanned to create a uniform sheet of light, while the intensity loss along the axis is compensated according to different specimens. Furthermore, multifocal generated in XZ plane can be used in multifocal multiphoton microscopy or optical trapping.

10070-27, Session 3

Bessel-beam illumination in a dual-axis confocal microscope reduces resolution degradation caused by refractive heterogeneities in tissues

Ye Chen, Adam K. Glaser, Jonathan T. C. Liu, Univ. of Washington (United States)

When performing laser-scanning microscopy in biological tissues, refractive heterogeneities (due to structures such as organelles and vessels) can cause spatial variations in the position (beam-steering) and shape (aberrations/distortions) of focused optical beams. These artifacts are known to deteriorate the image resolution of optical microscopes designed to visualize and monitor cellular structures in thick tissues at a large depth. While dual-axis confocal (DAC) microscopy exhibits improved optical-sectioning and spatial-filtering performance over traditional single-axis confocal microscopy, the image quality of DAC microscopes is particularly sensitive to refractive heterogeneities due to their use of off-axis illumination and collection beams that must intersect and focus at a common location (voxel) within tissues. In recent years, numerous groups have demonstrated that the propagation-invariant and self-reconstructing features of Bessel beams can benefit volumetric microscopy of tissues with refractive heterogeneities. Our studies have shown that Bessel beams display better positional stability and beam quality than Gaussian beams when propagating through media with micro-architectural heterogeneities. Here, we utilize both Gaussian and Bessel illumination in a point-scanned DAC microscope and quantify the resultant degradation in spatial resolution when imaging within optical phantoms, as well as when imaging fresh biological tissues. Results indicate that DAC microscopy with Bessel illumination exhibits less resolution degradation from microscopic tissue heterogeneities, compared to DAC microscopy with conventional Gaussian illumination.

10070-15, Session 4

Optical diffraction tomography of fluorescent distributions with single-pixel frequency-modulated imaging

Jeffrey J. Field, Randy A. Bartels, Colorado State Univ. (United States)

Fluorescent imaging is an indispensable component of many biological investigations. A challenge facing many researchers is the need to image biological tissues with high spatial resolution over large volumes. While fluorescent microscopes enable high resolution imaging, many with resolution below the diffraction limit, these tools are often restricted to imaging small volumes. In this contribution, we discuss an approach to achieve 3D imaging over large volumes of tissue while maintaining diffraction-limited spatial resolution. We utilize coherent holographic image reconstruction by phase transfer (CHIRPT), an imaging method that utilizes de-localized illumination intensity patterns, to collect optical diffraction tomography (ODT) images of fluorescent emission with a single-pixel detector. CHIRPT permits numerical reconstruction of 2D and 3D data sets by transferring the phase difference between two spatially-coherent illumination beams into temporal modulations of fluorescent emission. Since each fluorescent molecule in the illuminated region of the sample experiences a distinct temporal modulation pattern, the temporal signal collected from the single-pixel detector encodes the location of each fluorophore simultaneously. Unfortunately, a single CHIRPT image provides highly non-isotropic image resolution – diffraction limited in the lateral dimension, yet unsectioned in the axial dimension. By acquiring multiple views of the specimen at varied illumination angles, we show that it is possible to synthesize a single 2D view of the specimen with isotropic spatial resolution in the lateral and axial dimensions. We show 2D ODT images acquired with CHIPRT, and discuss methods to extend the principles of ODT-CHRIPT to 3D imaging with isotropic spatial resolution.

10070-16, Session 4

Spatially-controlled illumination with rescan confocal microscopy enhances image quality, resolution and reduces photodamage

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Fluorescence microscopy is an essential tool in bio-imaging. An inherent trade-off in fluorescence microscopy lies between image quality and photodamage. Recently, we introduced Re-scan Confocal Microscopy (RCM) as new technology with improved signal-to-noise ratio and lateral resolution by a factor of √2 as compared to standard confocal microscopy [1]. Prior to the introduction of RCM, we demonstrated that spatial control of illumination leads to reduced photodamage [2]. Here, we show that the combination of these two techniques leads to high-resolution imaging with reduced photodamage, without compromising image quality. Implementation of spatially-controlled illumination was carried out in RCM microscopy using a line scanning-based approach. By using a prediction algorithm, that uses information from the previously acquired line images, the spatial illumination profiles for the upcoming lines are decided, during imaging. As a proof-of–principle, we show images of convallaria obtained using RCM with reduced illumination dose without compromising image quality.

References:

[1] De Luca, Giulia MR, et al. “Re-scan confocal microscopy: scanning twice for better resolution.” Biomedical optics express 4.11 (2013): 2644-2656.

[2] Hoebe, R. A., et al. “Controlled light-exposure microscopy reduces

photobleaching and phototoxicity in fluorescence live-cell imaging." Nature biotechnology 25.2 (2007): 249-253.

10070-17, Session 4

Comparison of 3D structured patterns with tunable frequency for use in structured illumination microscopy

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In this contribution axially-localized high-contrast cosine patterns generated using a slit-prism illumination system based on either a Fresnel biprism or a Wollaston prism are compared in simulation. Similar structured patterns generated with wave interference have be used in struc-tured illumination microscopy (SIM) resulting in reconstructed final images with higher transverse resolution and optical-sectioning capability than conventional widefield microscopy. Our alternative methods to produce a 3D structured illumination pattern uses incoherent illumina-tion instead of the current approaches, which use coherent illumination, and are thus subject to coherence noise. In our approach, the prism (Fresnel biprism or Wollaston prism) is illuminated by a noncoherent emerging wave coming from a set of equidistant parallel slits which are inco-herently illuminated using quasi-monochromatic LED. It is important to mention that the Fresnel biprism has been previously demonstrated for generating 3D structured patterns. From the results reported using the Fresnel biprism, one can claim that the structured pattern created from a Fresnel bi-prism presents some advantages: (i) the spatial frequency of the 3D pattern can be easily tunable by moving the axial position of the biprism, and (ii) the phase-shifting of the 3D illumination pat-tern is performed easily by a lateral displacement of the Fresnel biprism. As we will show, the use of a Wollaston prism has two additional advantages. With this optical element, the 3D structured pattern shows larger lateral extension compared to the pattern obtained with the biprism, while the contrast of the fringes remains the same along the lateral coordinate dimension because it is not affected by an envelope function as in the case of the biprism.

10070-18, Session 4

Theoretical analysis of three-dimensional localization microscopy using metallic nanoslits for axial modulation

Taehwang Son, Changhun Lee, Donghyun Kim, Yonsei Univ. (Korea, Republic of)

We have investigated axial modulation of localized field that is induced at metallic nanoslit arrays by controlling light polarization in order to determine the distance between nanoslit and target molecules. Three-dimensional finite domain time difference method was used to calculate near-field distribution and the effect of polarization modulation. The wavelength of incident light was set to 488 nm. The nanoslit arrays were assumed to be of gold made on a glass substrate with period fixed at 0.5 ?m. The height and the fill factor of nanoslit were varied from 0.5 to 1 ?m and from 0.2 to 0.8, respectively. Overall, the maximum penetration length of near-field was obtained at the gap between nanoslits when light polarization was parallel to the direction of nanoslit, while perpendicular polarization was found to induce minimum penetration length and unlocalized evanescent wave over the nanoslit surface. It was shown that the electric field is confined between nanoslits due to the boundary condition in the case of parallel polarization. However, with perpendicular polarization, surface plasmon polariton, which propagates along the surface of gold nanoslit, is produced, thereby creating localized field at the edge to nanoslit. Fluorescent targets were assumed to be located above nanoslit and calibration curves with respect to the incident light polarization were obtained. The calibration curves were calculated using custom-built codes, the characteristics of which differ by the axial position of targets. The distinct difference between the curves shows the feasibility of determining or axially tracking the target position.

10070-19, Session 4

A state space based approach to localizing single molecules from multi-emitter images

Milad Rafiee Vahid, Jerry Chao, Texas A&M Univ. (United States); Elizabeth S. Ward, Texas A&M Health Science Ctr. (United States); Raimund J. Ober, Texas A&M Univ. (United States)

Single molecule super-resolution microscopy is a powerful tool that enables imaging at sub-diffraction-limit resolution. In this technique, subsets of stochastically photoactivated fluorophores are imaged over a sequence of frames and accurately localized, and the estimated locations are used to construct a high-resolution image of the cellular structures labeled by the fluorophores. Some localization methods are only suitable for analyzing frames in the sequence that contain sparse distributions of emitting fluorophores. These methods necessitate a relatively long experiment to ensure that enough frames with well-isolated fluorophores are acquired for the construction of the high-resolution image. To reduce the necessary number of frames, methods are available that can localize less well-isolated fluorophores in a given image. In general, however, these methods are sensitive to the selected region of interest, and require prior knowledge of the number of fluorophores in the given image and use of optimization algorithms with high computational complexity. We propose a fast method that is capable of localizing less well-isolated fluorophores without being encumbered with these drawbacks. The method models the given image as the frequency response of an n-order system obtained with a balanced state space realization algorithm based on singular value decomposition, and determines the single molecule locations as the pole locations of the resulting system. We also derive, in terms of the parameters of the resulting system, an expression for the high-resolution image constructed from the single molecule locations. We validate our method using both simulated and experimental multi-emitter images.

10070-11, Session PMon

Layer by layer visualization of light sheet microscopy data

Alessia Candeo, Cosimo D'Andrea, Gianluca Valentini, Andrea Bassi, Politecnico di Milano (Italy)

Light sheet fluorescence microscopy (LSFM) is a powerful tool to image biological specimens in depth at high resolution. It is commonly used to observe in-vivo the development and of embryos, plants and artificial tissues. Combined with chemical clearing it has been used to image entire mouse organs including mouse brain, spinal cord and lung.

However, a general problem of LSFM is that the huge amount of data generated in each acquisition produces unnecessary storage and processing overload, leading to major computational effort for localization and segmentation of anatomical features. Therefore, we developed an image processing routine to virtually "unfold" the sample and observe it layer-by-layer from its outer surface towards its center. This was achieved by a three step procedure consisting in: i) virtually unfold the 3D reconstruction of the sample; ii) observe it layer-by-layer; iii) identify distinct anatomical regions and statistically analyze multiple samples. We applied this method on specimens with different spatial scales, ranging from 100 um thick plant roots to few mm thick mouse organs. In particular, we studied the morphology of the mouse intestine, showing the external vasculature, the muscular layer and the mucosa over large portions of the organ. Using this approach, rather than analyzing the sample in a complex 3D geometry, we could image the samples layer by layer, facilitating the automatic segmentation and quantitative analysis of the sample.

10070-56, Session PMon

Investigation of spectrometer design for reducing roll-off in spectral-domain optical coherence tomography

Ana Doblas, Eric Boyers, Richard L. Blackmon, Amy L. Oldenburg, The Univ. of North Carolina at Chapel Hill (United States)

Spectral-domain optical coherence tomography (SD-OCT) is a biomedical imaging technique which produces depth-resolved images of refractive index heteroegeneity (via light scattering). In SD-OCT, the location and reflectance of scatterers in a sample are encoded in the spectrum of low-coherence light obtained by interfering light reflected from the reference and sample arms of an interferometer. The spectrum of the interfered light is then recorded with a spectrometer, which is composed of a diffraction grating, a lens, and a line scan camera, and the sample information is retrieved after digital signal processing. Although SD-OCT systems are widely used in biomedical applications, this method has the drawback that the signal-to-noise ratio (SNR) is reduced as the imaging depth increases (called "roll-off"). Given a fixed spectrometer wavelength range (which is typically set to capture the light source bandwidth), roll-off is determined by two terms: the spot size of the Gaussian beam at the sensor plane relative to the pixel size (i.e., the modulation transfer function, MTF), and the total number of pixels on the sensor. Here we investigate whether the first term, MTF, can be optimized with off-the-shelf lenses. Given a fixed f-number, we investigate which kind of lens produces less aberration for SD-OCT spectrometers and, consequently, minimizes the SNR fall-off. Several lens types are investigated, including plano-convex, biconvex, aspheric, and achromatic doublet. For each lens, we measure the MTF (i.e., the SNR as a function of the depth) after digital dispersion correction, and determine which lenses best mitigate roll-off in SD-OCT.

10070-57, Session PMon

Theoretical analysis of lateral resolution given by annular super-resolution phase plate

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In super-resolution microscopy (SRM) based on fluorescence depletion (FD), two color beams, i. e., pump and erase beams are used for illumination lights. To achieve SRM, the erase beam depletes fluorescence lights by quenching molecules with the first excited electronic state (S1) generated by the pump beam. When a donut-shaped erase beam is focused onto a dyed sample together with the Gaussian pump beam, a fluorescence spot on the sample shrinks remaining only the central region. Scanning the sample with this spot, we can obtain a fluorescence image with spatial resolution finer than the diffraction limit. A spiral phase plate (SPP) or an annular phase plate (APP) can be applied to generate the donut-shaped erase beam. Principally, the focused erase beam passing through APP has a three dimensional dark spot surrounded by lights near the focal region. Thus, the APP can provide three-dimensional SRM function. However, a hole size on the focal plane given by the APP is larger than that given by the SPP. Thus, it is believed that the APP cannot improve lateral spatial resolution like the SSP. Recently, a calculation using a vectoral optical model shows that the shape of the dark spot created by the APP becomes small as closing a numerical aperture (NA) to 1. In this study, we theoretically investigated lateral spatial resolution for SRM given by the APP in a case of a high NA objective lens based on a vectoral optical model.

10070-58, Session PMon

Relationship between reconstruction quality and scan type for compressive sensing based on cone beam CT reconstruction

Lin Zhang, Feng Gao, Huijuan Zhao, Limin Zhang, Jiao Li, Zhongxing Zhou, Tianjin Univ. (China)

There is direct evidence that the radiation doses associated with CT scans are associated with an increase in cancer risk. To reduce the radiation dose and simultaneously maintain the CT reconstruction quality, numerous algorithms have been proposed such as compressive sensing (CS) technique. CS theory asserts that one can recover certain signals and images from far fewer samples or measurements than traditional methods use. In this study, we mainly consider the relationship between the CT reconstruction quality and two undersampled scan types of CS technique, i.e., the sparse-angle scan and limited-angle scan. We also discuss the relationship between the CT reconstruction quality and the noise level of the projections, which is related to the time of radiation exposure. The results demonstrated that an appropriate selection of scan type of CS technique can effectively control the radiation dose with reduced exposure time.

10070-20, Session 5

Locating single molecules and cellular compartments in two and three dimensions *(Invited Paper)*

Md Tauhidul Islam, Jerry Chao, Texas A&M Univ. (United States); Elizabeth S. Ward, Texas A&M Health Science Ctr. (United States); Raimund J. Ober, Texas A&M Univ. (United States)

No Abstract Available

10070-21, Session 5

3D single molecule tracking with cellular context using remote focusing-multifocal plane microscopy

Dongyoung Kim, Sungyong You, Texas A&M Univ. (United States) and Texas A&M Health Science Ctr. (United States); Elizabeth S. Ward, Texas A&M Health Science Ctr. (United States) and The Univ. of Texas Southwestern Medical Ctr. at Dallas (United States); Raimund J. Ober, Texas A&M Univ. (United States) and Texas A&M Health Science Ctr. (United States)

Single molecule microscopy combined with 3D imaging enables the study of dynamic processes in living cells at the level of individual molecules. Multifocal plane microscopy (MUM) exemplifies such a modality, and has been shown to be capable of capturing the rapid subcellular trafficking of single molecules in thick samples by simultaneously imaging distinct focal planes within the sample. Regardless of the specific modality, however, the obtained 3D trajectories of single molecules often do not fully reveal the biological significance of the observed dynamics. This is because the missing cellular context is often also needed in order to properly understand the events observed at the molecular level. In principle, imaging of the cellular structures comprising the necessary context could be realized using the traditional approach to z-stack imaging. Such an implementation, however, is not suitable for single molecule imaging, since changing the focus of the microscope would also change the focal plane from which the

single molecules are imaged. Additionally, mechanical vibrations introduced by moving the objective lens or specimen stage might interfere with the imaging of the single molecule dynamics. Here, we make use of remote focusing to overcome these obstacles. We introduce the remote focusing-MUM (rMUM) modality, which allows 3D single molecule imaging with the simultaneous z-stack imaging of the surrounding cellular structures. Using rMUM, we demonstrate the 3D tracking of prostate-specific membrane antigen (PSMA) with a PSMA-specific antibody in a prostate cancer cell. The results provide a significantly more complete understanding of PSMA dynamics within the cellular environment.

10070-22, Session 5

Multiple-particle 3D tracking within 50nm localization and over 100μm range using temporal focusing two-photon microscopy

Yu Ding, Chunqiang Li, The Univ. of Texas at El Paso (United States)

Multiple-particle tracking in three dimensions is of great interest in the research of molecular dynamics and interactions in living cells. The spatial and temporal resolutions of the tracked particles are the key to the development of this technology. Here we present a method of three-dimensional multiple-particle tracking based on temporally focused two-photon microscopy. Defocused images of multiple particles are taken at video rate and in volumes of around 100?100?100 μm3. The z position information of each particle is encoded in the radii of the defocused images. Based on the images, algorithms are developed to reconstruct the 3D (x,y,z) positions of these particles. The long-term spatial localization precision can reach 50 nm in both axial and lateral dimensions with the help stage system stability. We demonstrate its capability of tracking living cells by videotaping microbes moving arbitrarily at speed up to 200 μm/s and reconstruct their trajectories in three dimensions. In addition, two-photon dual-color imaging is achieved by simultaneously exciting two types of fluorescent nanospheres mixed at Laser wavelength of 800 nm to demonstrate its potential applications on studies of molecular dynamics and interactions. This method provides a simple wide-field fluorescence imaging approach for deep multiple-particle 3D tracking. Furthermore, with an added activation/switching laser, this method can be developed into a three-dimensional super resolution microscopy.

10070-23, Session 5

Three-dimensional nanometre localization of nanoparticles using quantitative phase imaging: application to super-resolution microscopy

Pierre Bon, Ctr. National de la Recherche Scientifique (France)

Using the complementary intensity and phase images of light scattered by a metal nanoparticle, we achieve a nanoparticle localization precision of 1.5 nm in lateral and 6.5 nm in axial direction at a video rate of 50 frames per s. The accuracy can be pushed further to 0.7x0.7x2.7 nm3 while keeping the same temporal resolution by tracking two nanoparticles to get rid of the vibrations from the piezo stage and camera. The use of endogenous particles can also be considered to simplify the sample preparation: for example, a fixed vesicle inside the cell can be a good probe. This single-shot 3D nanoscale super-resolution is combined with dSTORM fluorescence imaging to enhance the image quality, improving both the spatial resolution and the signal-to-noise ratio. Our approach has several specific advantages.

It is implemented only using a commercial wavefront sensing device on a lateral port of the microscope: there is no complex holographic apparatus or moving elements; it is suitable to any microscope without major modifications, and the illumination wavelength can be easily tuned using regular filters to meet the user's requirements. As the method does not require a laser illumination, there is no speckle noise on the images. As it is not a fluorescent imaging approach, there is no photobleaching and the nanoparticle localization can be very fast with a large number of photons. Moreover, our approach can be used to stabilize super-resolution imaging systems when the sample is embedded in refractive-index-matching medium, as for in-depth STED imaging. Last, knowing the full scalar electromagnetic field allows to compute the propagation back to the best focus plane. This maintains an excellent super-localization precision even with a large and unknown defocus of several microns.

10070-24, Session 5

Self-interference nano-localization microscopy and maximum-likelihood estimation theory

Leanne Maurice, Alberto Bilenca, Ben-Gurion Univ. of the Negev (Israel)

Self-interference nano-localization microscopy is a method used to add into optical microscopes the third dimension with nanometer-level precision. The method has been demonstrated useful in super-resolving the optical axial (or depth) displacement of fluorescent and scattering light-sources with low-coherence emission [G. Shtengel et al., PNAS. 2009 106:3125-30; D. Aquino et al., Nat Methods. 2011; 8:353-9; E. Arbel & A. Bilenca, Sci Rep. 2015; 5:12560]. In this technique, the emission of a light-source is divided into two beams that are recombined prior to detection, producing self-interference patterns that conveys information on the optical axial displacement of the source. To retrieve this displacement information, three (four) phase-shift self-interference patterns are frequently used by introducing pi/3 (pi/2) phase shifts between the two beams, and the optical axial displacement of the light-source is then computed by a simple inverse tangent estimator.

In this talk, we propose a maximum likelihood (ML) approach for accurately estimating the axial displacement of point light-sources in self-interference nano-localization microscopy. Accounting for photon and background noise, an unbiased estimator for the emitter axial displacement will be derived and will be shown to be efficient, meaning it achieves the Cramer-Rao lower bound of axial displacement precision. Furthermore, we will discuss the superiority of the ML estimator over the simple inverse tangent estimator for recovering the emitter axial displacement along an extended depth range, and will describe the dependence of this superiority on background and photon noise levels. Performance evaluations using simulated and single-particle fluorescence experimental data will be presented.

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10070-25, Session 5

Simultaneous localization and Stokes polarimetry in a single image

Thomas G. Brown, Evan Espinoza, Univ. of Rochester (United States)

Particle localization is an important tool in microscopy; combining Stokes polarimetry with localization allows information about dipole orientation. It is known that a polarization dependent point spread function can allow complete Stokes polarimetry in a single irradiance image. When imaging particles using this method, it is necessary to simultaneously solve for particle localization and polarization. We describe a system and algorithm by which this can be accomplished for 2d localization and discuss the extension to 3d.

10070-26, Session 6

Label free nano-sensitive microscopy with sub-wavelength resolution

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For both the fundamental study of biological processes and early diagnosis of pathological processes, information about nanoscale tissue structure is crucial. The importance of high resolution imaging has been appreciated by Nobel Prize 2014 for the development of super-resolution microscopy. However, almost all known super-resolution techniques are based on use of specific labels that can destroy or lead to artificial results in living biological objects.

A novel label free contrast mechanism, based on the spectral encoding of spatial frequency (SESF) approach has been proposed recently. This approach permits to break the diffraction limit and dramatically improve resolution in the lateral direction for far field imaging. The idea of super-resolution SESF (srSESF) approach is to utilize the additional information about internal structure to resolve features within the object in the lateral direction. Experimentally demonstrated resolution was about 3 times better than diffraction limit of the optical imaging system.

Here we present a new version of srSESF approach which provides the possibility for comparison of different structures in time and space with nano-sensitivity to structural changes. We show that it is possible to resolve fine features, with separation in the lateral direction less than the diffraction resolution limit, via detecting a difference between the axial spatial frequency profiles of the numerically synthesized structure and the object's structure. We demonstrate that this contrast mechanism is very sensitive to structural alterations, and nanoscale changes in space and/or in time can be detected and visualized. Corresponding results of numerical simulation and experiments will be presented.

10070-28, Session 6

5D imaging approaches reveal the formation of distinct intracellular cAMP spatial gradients

Thomas C. Rich, Naga S. Annamdevula, Kenny Trinh, Andrea L. Britain, John R. Griswold, Joshua A. Deal, Chase Hoffman, Savannah West, Silas J. Leavesley, Univ. of South Alabama (United States)

Cyclic AMP (cAMP) is a ubiquitous second messenger known to differentially regulate many cellular functions. Several lines of evidence suggest that the distribution of cAMP within cells is not uniform. However, to date, no studies have measured the kinetics of 3D cAMP distributions within cells. This is largely due to the low signal-to-noise ratio of FRET-based probes. We previously reported that hyperspectral imaging improves the signal-to-noise ratio of FRET measurements. Here we utilized hyperspectral imaging approaches to measure FRET signals in five dimensions (5D) – three spatial (x, y, z), wavelength (?), and time (t) – allowing us to visualize cAMP gradients in pulmonary endothelial cells. cAMP levels were measured using a FRET-based sensor (H188) comprised of a cAMP binding domain sandwiched between FRET donor and acceptor – Turquoise and Venus fluorescent proteins. We observed cAMP gradients in response to 0.1 or 1 μM isoproterenol, 0.1 μM PGE1, or 50 μM forskolin. Forskolin and isoproterenol induced cAMP gradients from the apical (high cAMP) to basolateral (low cAMP) face of the cell. In contrast, PGE1-induced cAMP gradients formed from basolateral (high cAMP) to apical (low cAMP). Data suggest that 2D (x,y) studies of cAMP compartmentalization may lead to erroneous conclusions about the existence of cAMP gradients, and that 3D (x,y,z) studies are required to assess mechanisms of signaling specificity. Results demonstrate that 5D imaging technologies are powerful tools for measuring biochemical processes in discrete subcellular domains. This work was supported by NIH P01HL066299, S10RR027535, AHA 16PRE27130004 and the Abraham Mitchell Cancer Research Fund.

10070-29, Session 6

W-4PiSMSN: Ultrahigh resolution three dimensional imaging throughout whole cells

Fang Huang, Purdue Univ. (United States)

Major advances in biology have been tightly linked with innovations in microscopy. A major hurdle over the last ~100 years is the limited resolution of light microscopy. The advent of single molecule switching nanoscopy (SMSN, also known as PALM/STORM/FPALM) has overcome this fundamental limit by improving the resolution of fluorescence microscopy (250-?700 nm) by a factor of ten. This method routinely achieves 20-?40 nm lateral resolution and 50-?80 nm resolution in the axial direction. While the inferior axial resolution largely restricts the biological discoveries to two?-dimensional observations, interferometric SMSN (iPALM or 4Pi?SMSN) achieves unprecedented 10 to 20 nm axial resolution by coherently combining single?-molecule emissions in a two opposing-?objective setup. It allowed, for the first time, the molecular anatomy of focal adhesions to be mapped with nano-meter precision. However, the physical principle of 4Pi geometry limits this approach to isolated structures in thin samples because the single molecule interference pattern repeats every 250 nm in depth. While most of biological processes happen deep in the cellular volume, driving ultra-?high resolution imaging deeper into the cell will lead to a new wave of biological discoveries.

Here, I present our recent progress on whole?-cell 4Pi?SMSN resulting from the confluence of multiple innovations. This system, for the first time, allowed super-?resolution imaging of a ~10 μm thick sample using 4Pi geometry achieving 10-?15 nm resolution throughout the depth. This resolution is 20?-50 times higher than conventional microscopy with imaging depth improved by 10-?40 fold from the state of art technology of interferometric SMSN. It enables ultra-?high resolution three?-dimensional imaging for vast majority of the subcellular structures. We demonstrate applications in a range of delicate cellular structures including: bacteriophages, ER, mitochondria, nuclear pore complexes, primary cilia, Golgi complex-?associated COPI vesicles, and synaptonemal complexes in whole mouse spermatocytes.

10070-51, Session 6

Optimized point spread function for 3D super-resolution imaging of continuous objects

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We present a new point spread function (PSF) design that is optimal for super-resolving fluorescent continuous objects using expanded point information content (EPIC) microscopy. The PSF is optimized within a predetermined extended depth range by minimizing least square-errors between the modeled PSF and the acquired images. By using the designed PSF with focused spot excitation and numerical optimization, we can super-resolve features beyond the diffraction limit without the need for special fluorophores or structured illumination. We believe that our results present an insight into a new approach to 3D super-resolution microscopy with a

simple instrument design that is well suited for potential high-speed 3D imaging.

10070-31, Session 7

Interleaved image reconstruction for structured illumination microscopy

Ying Ma, Kaiqin Chu, Univ. of Science and Technology of China (China); Deanna Wolfson, Norut Tromsø (Norway); Zachary J. Smith, Univ. of Science and Technology of China (China)

One important advantage of structured illumination microscopy over other super resolution (SR) microscopy techniques is its ability to image fast. However, it still requires multiple exposures to get a single super-resolution frame. In each exposure, the illumination pattern shifts or rotates. Those changes require certain time between frames. In particular when those changes are induced by mechanical movement, the movement time becomes a limiting factor in imaging speed. Meanwhile for each frame the exposure has to be high enough to achieve a certain signal level for the reconstruction result to be acceptable. Overall these factors limit the practical SR frame rate to be around 1fps. This is still too slow for many biological dynamics, such as neuron activities. In SR microscopy multiple subimages are required to reconstruct one single SR frame. For example, 9 subimages are required for a single 2D SR image. Typically these subimages are not shared among time points and each subimage is used in the reconstruction of only a single frame. However, if there is no delay between time points in a time lapse, we can use subimages from two consecutive time points to create a new SR frame. The new SR frame images the activity between those two time points. Thus, we create a new SR frame by interleaving subimages from neighboring time points. Here we demonstrate that this improves the SR frame rate by up to three fold without changing exposure times or the reconstruction quality.

10070-32, Session 7

Three-dimensional resolution enhancement for deep-tissue imaging

I-Cheng Su, Kuo-Jen Hsu, Po-Ting Shen, National Taiwan Univ. (Taiwan); Yen-Yin Lin, National Tsing Hua Univ. (Taiwan); Shi-Wei Chu, National Taiwan Univ. (Taiwan)

Optical microscopy is key to biological researches since it allows non-invasive and very specific imaging modalities in imaging cells and tissues. Nevertheless, in a deep tissue, these methods have to compromise with resolution due to not only diffraction but also scattering. Although several resolution enhancement methods have been developed during the last decade, different techniques suffer from different limitation. For example, STED require strong illumination; PALM and STORM are relatively slow. Furthermore, current superresolution methods cannot be used for deep tissue because of the distortion in either the illumination or the collection path. Other than pushing spatial resolution with hardware design, there are continuous efforts to improve resolution by software analysis, with the advantages of minimizing potential photo-damage and avoiding long acquisition time. Deconvolution, a conventional computational method, achieves resolution enhancement with a priori point-spread-function (PSF) knowledge. However, since the PSF is difficult to determine in a deep tissue due to multiple scattering and refraction, deconvolution does not function effectively when the imaging deeper than one transmission mean free path. In this study, we introduce a new computational method, three-dimensional virtual spatial overlap modulation microscopy (3D-vSPOM). Compared to deconvolution, this new approach provides not only much better resolution enhancement for deep tissue imaging, but also greatly improved noise-immune response. Our method is ready to combine with two-photon or other nonlinear microscopy to achieve fast and high-resolution 3D imaging.

10070-34, Session 7

Imaging beyond scattering limits utilizing ARF as a guidestar

Ali Vakili, Northeastern Univ. (United States); Joseph L. Hollmann, ICFO - Institut de Ciències Fotòniques (Spain); Ray Glynn Holt, Boston Univ. (United States); Charles A. DiMarzio, Northeastern Univ. (United States)

The Main challenge in optical imaging in a highly scattering medium like soft tissue is the multiple scattering. Light that travels through such medium undergoes several scattering events and can not provide spatially resolved information. In the case of soft tissue, acoustic waves can travel through the medium with negligible scattering. Therefore, several methods such as Acousto-Optical imaging modalities have been introduced to push the optical imaging beyond the scattering limits. We have previously shown that a focused acoustic wave produces a Guidestar by generating acoustic radiation force (ARF) and speckle pattern analysis can provide information about the displacement induced by the ARF in the focal spot of the acoustic wave. Simulation results obtained computationally by utilizing fixed particle Monte-Carlo method, show that when particles move as the result of the ARF generation inside the medium, the phase of the interfering pattern and therefore, the resulting speckle pattern changes. The spatial average in the changes in the speckle pattern can provide information about mechanical and thermal properties from the acoustic focal spot. The mechanical and thermal properties can be distinguished by the time scale of the changes. Hence, by scanning the focused acoustic wave within the medium, this method can potentially provide an image based on the mechanical properties of the medium. The resolution of the image is limited by the size of the focal spot of the focused acoustic wave and the contrast is governed by the changes in the optical path length of the interfering waves.

10070-35, Session 8

Fast multi-plane functional imaging combining acousto-optic switching and remote focusing

Ee Zhuan Chong, Ines Barreiros, Bei Li, Michael Kohl, Martin J. Booth, Univ. of Oxford (United Kingdom)

Neural signalling is not restricted to two-dimensional network structures but extends further into three-dimensional volume. Fast volumetric imaging is therefore clearly preferable to the conventional two-dimensional scanning mode. To get a more complete picture on the dynamics of the neuron-to-neuron interactions, we have developed a pseudo-parallelised multi-plane two-photon excitation imaging system through the incorporation of acousto-optic switching and a remote focusing technique into a resonant scanning microscope. This permits the recording of millisecond scale fluorescence transients of calcium indicators from large populations of neurons upon neural firing events at multiple chosen axial planes in very short time frame. While the remote focusing system offers aberration-free axial scanning over a few hundreds of micrometres of depth, the acousto-optic deflector provides high speed optical switching between different laser beam paths in sub-microsecond timescale which, in turn, controls the axial focal plane to be targeted. Here, we report on the development of the high temporal multi-plane targeted microscope and its potential application in the calcium imaging of activity in the mouse brain in three dimensions.

10070-36, Session 8

Fast axial scanning for intravital 2-photon microscopy using liquid lens technology

Kayvan F. Tehrani, Luke J. Mortensen, The Univ. of Georgia (United States)

Scanning microscopy methods require movement of the focus in Z coordinates to produce an image of a 3-dimensional volume. In a typical imaging system, the optical setup is kept fixed and either the sample or the objective is translated with a mechanical stage driven by a stepper motor or a piezoelectric element. Although mechanical Z scanning is precise; its slow response and vulnerability to mechanical vibrations and stress make it disadvantageous. This is especially important when a dynamic, time-varying sample, such as a live cell structure is being imaged. An alternative method less susceptible to these problems is to change the focal plane using conjugate optics. Deformable mirrors, acousto-optics, and electrically tunable lenses have been experimented with to achieve this goal and have attained very fast and precise Z-scanning without physically moving the sample. In this paper, we are presenting the use of a liquid lens for fast axial scanning. Liquid lenses have a long functional life, high degree of phase shift, and low sensitivity to mechanical stress. They work on the principle of refraction at a liquid-liquid interface. At the boundary of a polar and an apolar liquid, a spherical surface is formed whose curvature can be controlled by adjusting its relative wettability using electro-wetting. We characterize the effects of the lens on attainable Z displacement, beam spectral characteristics, and pulse duration as compared with mechanical scanning. We show 4D and oblique plane imaging of cells inside the bone marrow of mice at 30 frames per second.

10070-37, Session 8

Rejecting scattered light using orthogonally encoded structured illumination

Vicente Parot, Harvard Univ. (United States) and Massachusetts Institute of Technology (United States); Yoav Adam, Samouil Farhi, Shan Lou, Simon Kheifets, Harvard Univ. (United States); Adam E. Cohen, Harvard Univ. (United States) and Howard Hughes Medical Institute (United States)

Fluorescence microscopy in the brain and other turbid tissues is affected by scattered light and off-focus fluorescence. These factors increase the background and noise level of images and ultimately limit the imaging depth. We devised a novel method to perform optical sectioning with rejection of scattered light in a wide-field 1-photon microscope. A digital micromirror device (DMD) projects patterned illumination onto the sample. The patterns comprise a permuted Hadamard coding matrix arranged in a repeating spatial period, so neighboring sample locations are illuminated in orthogonal functions of time. A custom reconstruction algorithm assigns detected photons to their source location on the basis of their temporal encoding. This approach is insensitive to scatter of the emitted photons, though scattering of incident photons can degrade the resolution. In conventional structured illumination microscopy (SIM), the periodic illumination can create aliasing image artifacts. Hadamard imaging, in contrast, uses pseudorandom patterns that have a uniform spectral density and thus lead to a sharp point spread function in the reconstructed image. We demonstrate Hadamard imaging in the mouse brain, and in phantoms with varying scattering and fluorescence distributions. We compare the performance of Hadamard and conventional SIM imaging.

10070-38, Session 8

Accurate time-domain angular jitter measurements for a high-speed polygon scanner

Youn Young Ji, Byunghwy So, Wonsang Hwang, Dongeun Kim, Junwoo Kim, Dugyoung Kim, Yonsei Univ. (Korea, Republic of)

A laser scanning system is a key component for a scanning confocal microscopy, laser scanning display, and laser printers, where a laser beam is deflected very fast by a scanning system. Scanning speed, linearity, stability or repeatability, and scanning range are important parameters for a good laser scanner. Even though a Galvanometer is a well-adapted conventional laser scanner, it is hard to increase the scanning speed more than 4 kHz due to the mass and the size of an oscillating mirror. Nonlinear scanning angle due to the oscillating motion of a mirror is another drawback of a Galvanometer scanner. Polygon mirror scanners, acousto-optical modulators, electro-optical modulators, and MEMS mirrors are alternative scanning components which can be used for a confocal microscopy. Because of its cheap prices, linearity in scanning angle, and fast scanning speed, a rotating polygon mirror scanner is the most popular solutions for high-speed confocal imaging. Since any unequal angles or asymmetry in a polygon mirror surface produce a periodic scanning jitters in the time-domain, it is very important to measure timing jitters associated with unequal angles of mirrors in a polygon scanner. In this paper we propose an effective time-domain measurement method which can characterize the scanning angle jitters for a polygon mirror. Our setup includes a 635 nm wavelength semiconductor laser, photodiode, two lenses, and a high-speed digitizer. A polygon scanner with 12 facets were tested with a rotating speed of 500 Hz. The accuracy of measured angles for this polygon mirror is presented.

To detect the signal of the photodiode, we use high speed digitizer which have 2Ga/s sampling rate with 256MB memory. We obtained photodiode signal 12 mirror facet at one shot. In these sequences, angle jitter was successfully calculated from the data.

10070-45, Session 8

Micromechanical actuation of elastic optical needle-like probes for improved field of view in tissue imaging

Ismail Emre Araci, Santa Clara Univ. (United States); Eric E. Sabelman, Kaiser Permanente Medical Ctr. (United States)

Deep brain stimulation (DBS) is used for the treatment of various movement disorders including Parkinson's disease. The efficacy of the DBS technique can be improved as well as risks that are associated with DBS can be reduced by better electrode positioning methods. A front-looking OCT imaging probe attached to a flexible fiber-optic cable of the same dimensions as the DBS electrodes are needed for better positioning of DBS electrodes. DBS electrodes could be placed in the same stereotactic guide tubes. A forward-looking OCT probe could provide the first exploration along a planned trajectory, followed by a microelectrode for somatotopic signal recording as at present, and then a third side-looking OCT probe to identify nearby structures that might alter stimulating current flow. This sequence is optimal because both forward-looking and recording probes work best with undisturbed tissue deep to the sensor tip, while side-looking OCT is insensitive to tissue damage directly on the probe axis. While side-looking needle-like probes for 3D imaging are available, building the front-looking probes with same performance remain to be a challenge. In this study, we present the design and preliminary test results of an optofluidic needle-like probe which is attached to a fiber optic cable and can be used for 3D imaging of the brain.

Often the source of epileptic seizures is in the cortex rather than deep brain structures. Electrical mapping of seizure foci is done with low-spatial-resolution surface electrode grids, which provide minimal guidance for resection of dysplastic tissue and consequent risk of damage to the normal cortex. OCT probes such as envisioned for DBS held either in the same stereotactic apparatus or in a simpler probe holder combined with infrared camera navigation could map boundaries of dysplastic tissue in both area and depth to about 10 mm. Dysplastic cortex is known to be distinguishable from normal tissue. While the mechanism for positioning a cortical OCT probe may differ from DBS-specific designs, the same OCT system can be used to test both applications.

10070-39, Session 9

White matter segmentation by estimating tissue optical attenuation from volumetric OCT massive histology of whole rodent brains

Frédéric Lesage, Ecole Polytechnique de Montréal (Canada) and Institut de Cardiologie de Montréal (Canada); Joel Lefebvre, Alexandre Castonguay, Ecole Polytechnique de Montréal (Canada)

Whole rodent brains are imaged using an automated massive histology setup combined with an Optical Coherence Tomography (OCT) microscope. Thousands of OCT volumetric slices are acquired for a brain, each covering a size of about 2.5x2.5x0.8 mm3 with a resolution of 4.9x4.9x6.5 microns. The tile positions within the mosaic are evaluated using a displacement model of the motorised stage and pairwise coregistration. Volume blending is determined by solving the 3D Laplace equation with Dirichlet boundary conditions, and consecutive slices are assembled using the cross-correlation of their 2D image gradient. This reconstruction algorithm results in a 3D map of optical reflectivity for the whole brain at micrometric resolution.

The OCT reflectivity contrast of myelinated axons depends on their relative orientation with respect to the microscope optical axis. In turn, the optical attenuation coefficient of the tissue has a reduced dependence on orientation when compared to reflectivity. We exploited this feature to estimate the local attenuation coefficient. The algorithm first compensates for the depth-dependent contrast of the microscope's PSF. A Beer-Lambert law is then fitted on each A-Line portions and the attenuation coefficient is estimated from this fit. The attenuation map obtained exhibits a high contrast for all white matter fibers regardless of their orientation. This new method to measure the tissue optical attenuation from the intrinsic OCT reflectivity contributes to better white matter tissue segmentation. The 3D maps of reflectivity and attenuation are combined to study the white matter at a microscopic scale for the whole brain.

10070-40, Session 9

Comparison of two structured illumination techniques based on different 3D illumination patterns

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Manipulating the excitation pattern in optical microscopy has led to several super-resolution techniques. Among different patterns, the lateral sinusoidal excitation was used for the first demonstration of structured illumination microscopy (SIM), which provides the fastest SIM acquisition system compared to the multi-spot illumination approach. Moreover, the 3D patterns that include lateral and axial variations in the illumination attract more attention recently as they address resolution enhancement in three dimensions. A three-wave interference technique in SIM has been developed to provide super-resolution and optical sectioning based on monochromatic illumination, which is subject to speckle and coherent noise [1]. In this paper, we investigate a novel tunable technique that creates a 3D pattern from an incoherent source. It uses a set of two incoherently illuminated parallel slits that acts as the light source for a Fresnel biprism. The setup is able to modulate the illumination pattern in the object space both axially and laterally with adjustable modulation frequencies [2,3]. The 3D forward model for the new system is developed to consider the effect of the axial modulation within the 3D patterned illumination. The performance of 3D-SIM based on the three-wave interference and the two-slit biprism setup are investigated in simulation and compared based on two different criteria. First, restored images obtained for both 3D-SIM systems using the generalized Wiener filter are compared to determine the effect of the illumination pattern on the reconstruction. Second, the effective optical transfer function (OTF) of both systems is studied to determine the axial and lateral resolution enhancement that is obtained in each case.

10070-41, Session 9

Single image sectioning using spatial frequency domain imaging

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Structured illumination microscopy (SIM) works well to produce depth information by removing undesired light from out of focus planes within a specimen. However, it generally requires multiple images in order to produce sectioning. We show that, under the right conditions, we can leverage Spatial Frequency Domain Imaging (SFDI) to produce both sectioning and relative depth information about a specimen using only a single image. First, an image of the specimen is captured, modulated by a discrete spatial frequency. From this image, the unmodulated (DC) image must be estimated. Due to this estimation, we require that the modulation depth be less than 1, to ensure that there are no zeros within the image. After processing, we find that the axial resolution is comparable to that of traditional 3 phase SIM. However, we find that there is a slight degradation in lateral resolution, related to errors in the estimation of the DC image. Multiple targets with various depth and opaqueness are considered and compared to traditional SIM. From these data, we are able to show that this methods of sectioning works at depth, providing a quick and useful way to produce non-invasive sectioning in real-time. This would be particularly useful in applications such as skin cancer detection and tumor margin analysis, among others. Additionally, we find that measuring the DC image independent of the modulated image produces sectioning with no degradation in axial resolution. With only two images, it is possible to produce nominally equivalent sectioned images to that of the traditional 3 image SIM.

10070-42, Session 9

Local and biorthogonal wavelet-based noise removal technique for three-dimensional image reconstruction

Ying Wang, Hanh N. D. Le, Jin U. Kang, Johns Hopkins Univ. (United States)

Obstructive sleep apnea (OSA) during sleepis caused by full or partial obstructions of the upper airway and a three-dimensional (3D) reconstruction of mouth inner structures is necessary for the assessment of OSA. Due to the tissue optical characteristic under low light environment, a 3D reconstruction of mouth structure suffers from unwanted signals from shot noise, dark current noise and Fresnel reflection. Here we propose a noise-removal method for the processing of 3D reconstruction using multi-fringe profilometry method with aims of retaining phase information while eliminating high intensity noise in the fringe images. Firstly, local speckle noises are detected by thresholding and interpolated with the neighboring pixel intensity. Secondly, the bright point noises are detected based on a weaker thresholding only in fringe direction and interpolated with the vertical neighboring pixel intensity to avoid phase information destruction. Thirdly, a biorthogonal wavelet decomposition was implemented for noise removal in the low frequency images. To demonstrate our method four pattern fringes of the tongue and tonsil images were processed. The results show high intensity noises were removed and phase information with diffuse reflection are restored, the 3D reconstruction accuracy are improved with the noises removed images compared to the 3D reconstruction result with the original images.

10070-43, Session 9

Time-resolved wavelet-based acquisitions using a single-pixel camera

Florian Rousset, CREATIS (France) and Politecnico di Milano (Italy); Nicolas Ducros, CREATIS (France); Andrea Farina, CNR-Istituto di Fotonica e Nanotecnologie (Italy); Gianluca Valentini, Cosimo D'Andrea, Politecnico di Milano (Italy); Françoise Peyrin, CREATIS (France)

Time-resolved imaging plays an important role for biomedical applications such as Fluorescence Lifetime Imaging Microscopy (FLIM) which enables to study the environment of fluorophores, for instance, through variation of pH or refractive index. Generally a tradeoff between the temporal and spatial resolution of the measurements has to be found. Moreover, a high spatial and/or temporal resolution leads to an increase of the acquisition time.

We propose to couple a Single-Pixel Camera (SPC) with a unique Time Correlated Single Photon Counting (TCSPC) board in order to obtain a time-resolved imaging system having high both spatial and temporal resolutions. As an alternative to Compressive Sensing (CS), we recently suggested a wavelet-based acquisition strategy that enables the image to be restored directly, disposing of the computational overhead of l1-minimization induced by CS. Our acquisition strategy is adaptive and allows high compression rates with little degradation of the image quality.

We demonstrate the applicability of our approach to fluorescence lifetime imaging. In particular, we show experimental results obtained by imaging samples embedding fluorophores with different lifetimes. A stack of images from each temporal gate can be obtained and a fitting algorithm can be employed to recover the lifetime map of the imaged object.

In conclusion, coupling the SPC with a TCSPC and a wavelet-based approach permits to obtain a simple, faster and inexpensive yet efficient time-resolved imaging system. Such a setup can easily be transposable on a microscope in order to perform FLIM measurements.

10070-44, Session 10

Multi-beam optical coherence tomography for microvascular imaging of human skin in vivo

Chaoliang Chen, Ryerson Univ. (Canada)

In this paper, a multi-beam optical coherence tomography (OCT) was used to reconstruct the microvascular image of human skin in vivo with phase resolved Doppler OCT (PRDOCT), phase resolved Doppler variance (PRDV) and speckle variance OCT (svOCT), in which the blood flow image was calculated by averaging the four blood flow images obtained by the four beams. In PRDOCT method, it is difficult to detect the blood flow perpendicular to optical axis of the probe beam for single beam OCT, but the multi-beam scanning method can solve this because the input angles of the four probe beams are slightly different from each other. The results of the in vivo skin imaging on a healthy volunteer demonstrate that, in PRDOCT method, the blood flow signals don't appear uniformly across the four images obtained by the four beams individually and all the blood flow signals can be seen in the averaged images, furthermore, the signal-to-noise ratio (SNR) of the blood flow signals can be improved by the multi-beam scanning method within the all three algorithms mentioned above. At last, by scanning a patient with non-melanoma skin cancer, it is demonstrated that the multi-beam scanning method can offer the microvascular images with lower noise floor, which is more helpful for disease diagnosis in clinical application.

10070-46, Session 10

Ultra-broadband k-space spectrometer for spectral domain optical coherence tomography (SD-OCT)

Gongpu Lan, Guoqiang Li, The Ohio State Univ. (United States)

In spectral domain optical coherence tomography (SD-OCT), high performance spectrometer is required for ultrabroad-band light source (which enables high axial resolution) and low sensitivity fall-off (which ensures high signal sensitivity in an effective imaging depth). A key factor responsible for the sensitivity fall-off is the nonlinear spectral distribution of wavenumber (k) in conventional spectrometer, where a grating is used as the dispersion component. We report a linear-in-k (k-space) spectrometer, based on the combination of a diffraction grating and an isosceles prism as the dispersion component, for an ultrabroad bandwidth (760 nm ~ 920 nm) SD-OCT. The dispersion linearity in k space is optimized and the optical path differences are minimized via ray tracing. The dispersed point spread functions (PSFs) are shaped to fit the rectangular pixel size of the line camera — aiming to improve the sampling rates of the pixels. An experimental SD-OCT has been built up to measure the sensitivity fall-off utilizing this spectrometer. Results show that this k-space spectrometer can reduce the angular nonlinearity error in k from 14.86% to 0.47% (by approximately 30 times!) compared to conventional spectrometer. Although the RMS spot diameters are of great difference (2.8 ?m ~ 12.1 ?m) across the spectrum, the sampling rate by pixel (14 ?m ? 28 ?m) are very similar (82.12% ~ 89.10%) due to the shape-fit of PSFs to pixels. The test result shows a drop in sensitivity as -8.4 dB at the maximum depth of 2.3mm.

10070-47, Session 10

The biodynamic microscope: Doppler spectroscopy of subcellular motion inside 3D living tissue

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Biodynamic imaging is a novel 3D optical imaging technology that uses short-coherence dynamic light scattering to measure the intracellular motions of cells inside their natural microenvironments This imaging approach is label-free and non-invasive and probes up to 1 mm deep inside three-dimensional living tissue. We have developed a biodynamic microscope by integrating a biodynamic imaging module into a commercial Olympus microscope. The microscope retains its conventional imaging channels for the user, while adding a biodynamic channel for functional 3D imaging. The biodynamic module resides in one of the two optical expansion decks of the microscope beneath the nosepiece. The module is monolithically machined from a block of aluminum to maintain high interferometric stability. The information content of biodynamic imaging is captured through tissue dynamics spectroscopy that captures the changes in the Doppler signatures from intracellular constituents through fluctuation spectroscopy. The Doppler beats of all the intracellular components produce a fluctuating intensity that encodes the individual Doppler frequencies. The associated dynamic intracellular mechanisms include organelle transport, membrane undulations, cytoskeletal restructuring, strain at cellular adhesions, cytokinesis, mitosis, exo- and endo-cytosis among others. The imaging contrast is based on statistical optics and ensemble interference with a resolution voxel size of approximately 10 microns, providing 3D tissue-scale maps of intracellular activity. The development of the 3D biodynamic microscope can become a critical new tool for assessing efficacy of drugs and the suitability of specific types of tissue growth for drug discovery and development.

10070-48, Session 11

Raman scattering, phase imaging, and nanoparticle-enhanced Raman analysis as methods to probe immune cells *(Invited Paper)*

Nicolas Pavillon, Alison J. Hobro, Nicholas I. Smith, Osaka Univ. (Japan)

No Abstract Available

10070-49, Session 11

A mosaicing approach for optical diffraction tomography

Seth D. Smith-Dryden, Shengli Fan, Guifang Li, Bahaa E. A. Saleh, Univ. of Central Florida (United States)

Optical diffraction tomography (ODT) is a technique for imaging weakly scattering phase objects. In ODT, the phase object is illuminated by a plane wave from multiple directions about a center, and the corresponding diffracted optical fields are measured. The object is then reconstructed using algorithms such as the direct Fourier interpolation, filtered back propagation, or filtered back projection. While ODT potentially offers high-resolution reconstruction capabilities, many commonly used inversion techniques assume that distances from points on the sample to the detector do not change when viewed from different angles. This condition is only satisfied for points near the center of rotation, and so these points are reconstructed more accurately in the inversion process than points further away from the center. To address this issue, we develop a new two-step ODT inversion technique. First, using a single ODT measurement, we individually reconstruct small regions around a set of virtual centers of rotation throughout the sample. Then, by mosaicing together these regions, the fidelity of reconstruction becomes uniform throughout the sample. We have validated this technique by performing inversions on various sets of scattering cylinders, for which previously off-axis features failed to reconstruct using conventional inversion techniques.

10070-50, Session 11

Structured illumination for combined 3D quantitative phase and fluorescence sub-diffraction microscopy

Shwetadwip Chowdhury, Joseph A. Izatt, Duke Univ. (United States)

In the biological sciences, sub-diffraction resolution imaging plays a crucial role in the high-resolution visualization of sub-cellular structures. Unfortunately, due to the fundamentally different image formation processes involved in phase (coherent) versus fluorescent (incoherent) imaging, a generalized sub-diffraction imaging technique has been difficult to find. This poses a challenge for biologists, who typically have to choose between either unimodal sub-diffraction or multimodal diffraction-limited imaging. This hinders analysis that synergistically combines the molecular-specific, background-free imaging capabilities of fluorescence microscopy with the endogenous-contrast, biophysical and biochemical insights from light-scattering or quantitative-phase (QP) microscopies at high-resolution, sub-diffraction size scales. We experimentally show, for the first time to our knowledge, that structured illumination (SI) allows multimodal sub-diffraction resolution imaging using both fluorescent and QP contrast. We introduce a novel, high resolution optical system that combines conventional SI-microscopy with SI-diffraction-phase-microscopy, and demonstrate its multimodal sub-diffraction imaging capabilities for both quantitative-phase (QP) and fluorescent imaging. Our optical system uses 3-beam illumination, which allows multimodal 3D sub-diffraction capabilities, and experimental results show increase in lateral and axial resolutions for both fluorescent and QP imaging. To demonstrate the potential of this technique, we demonstrate sub-diffraction resolution, multimodal SI imaging of A549 cells fluorescently stained for F-actin, such that the QP and fluorescent signals may offer unique, but complementary, insights into the biological samples.

10070-52, Session 11

Robust phase unwrapping for phase images in Fourier domain Doppler optical coherence tomography

Yong Huang, Shaoyan Xia, Shizhao Peng, Yanfeng Wu, Xiaodi Tan, Beijing Institute of Technology (China)

To solve 2ϖ ambiguity of the phase images in the Fourier domain Doppler optical coherence tomography (FDOCT), we introduce a modified network programming technique to FDOCT for the first time to the best of our knowledge. The proposed method presents an assumption that the error of the discrete derivatives between the unwrapped phase and wrapped phase can be arbitrary values instead of integer-multiple of 2ϖ in network programing method. Thus wrapped images can be restored with great accuracy and robust noise tolerance. To prove this argument, we compared our method with other common fives methods (Goldstein, least-square with FFT, synthetic wavelength, path-independent using TV denoising, network programming). Experimental study on simulated images, phantom OCT images and real vessel OCT images were performed. Parameters including root mean-square error (RMSE) between true image and the unwrapping image and noise amplification degree (NAD) were adopted to compare different methods. Our method consistently achieves the optimal results among all these methods.

10070-59, Session 11

Synchronous 3D (plus time) cardiac imaging in embryonic zebrafish using light field microscopy with selective volume illumination

Sara Madaan, Thai V. Truong, Daniel B. Holland, Scott E. Fraser, The Univ. of Southern California (United States)

Quantitative analysis of blood flow in the beating heart during early stages of development is essential in order to understand the role physical forces, such as shear stress, play in endothelial cell alignment, cardiogenesis, and cardiac disease formation. However, acquiring 3D (plus time) information about blood flow in the beating heart is a fundamental challenge because of the 2D nature of the detectors and the rapid non-cyclical motion of blood cells.

In this paper we present an imaging modality based on Light Field Microscopy (LFM) that enables the acquisition of velocity flow fields in the zebrafish heart over time frames ranging from a few hours to several days.

10070-53, Session 12

Dynamic multiphoton multicolor imaging of the infected mouse lung

Daniel Fiole, Institut de Recherche Biomédicale des Armées (France) and Institut Pasteur (France); Pierre Deman, Institut de Recherche Biomédicale des Armées (France); Jean-Nicolas Tournier, Institut de Recherche Biomédicale des Armées (France) and Institut Pasteur

(France) and Ecole du Val-de-Grâce (France)

Given the very large interface it offers toward the outside environment, the lung has developed a highly efficient and balanced immune system which maintains balance between inflammation and tolerance. This efficient system involves numerous cellular protagonists, whose coordinated behavior is far from being fully understood. This lack of knowledge can be attributed to both the difficulty inherent to imaging an organ whose physiology is intrinsically linked to motion, and, in the case of fluorescence microscopy, to the complex setup required for multicolor imaging.

The protocol herein presented address both these issues. First, we describe a technique which enables dynamic ex vivo imaging of lung sections kept in warm refreshed medium in order to maintain cell dynamics for up to two hours. The main improvement is that the technique does not require any instillation of agarose into the lungs, in order not to interfere with the normal behavior of alveolar cells.

Then, we share a two-photon–based experimental design allowing 6-color imaging of the infected lung using two imaging modalities. It simultaneously targets dendritic cells, monocytes, alveolar macrophages, neutrophils, and inhaled bacteria using the two-photon excitation of purposely chosen fluorophores, and fibrillar collagen using the second harmonic generation.

To our knowledge, this protocol is the first allowing simultaneous imaging of four main immune cell populations together with bacteria and alveoli at the infected lung level. As such, it constitutes a powerful new tool for immunologists interested in the infection-driven dynamic interplay of pulmonary immune cells.

10070-54, Session 12

Quantification of morphological image features of AOM-induced dysplasia in murine colorectal tissue

Sandra P. Prieto, Haley M. James, Timothy J. Muldoon, Univ. of Arkansas (United States)

Morphologic changes in epithelium and collagen structure have been studied for various types of cancer, and while studies have shown a correlation between collagen density, epithelial morphology, and the prevalence of genetic mutations, the quantification of crypt morphology in colorectal cancer has not been fully explored. Second harmonic generation (SHG) imaging of collagen has shown decreased collagen density in various tumor tissues, such as breast and cervical cancer, and differences in fiber orientation. In order to better understand the correlation between dysplasia and collagen structure, we used SHG images to investigate the differences in dysplastic tissue morphology, as well as quantify the collagen density and orientation in dysplastic colorectal tissue. We acquired images of freshly resected label-free ex vivo murine colorectal tissue from mice 10, 14, and 18 weeks following a course of azoxymethane injections. Images were acquired with a 20x (0.8 NA) objective, and a 520μm field of view. SHG imaging was performed using two-photon 820nm excitation, at 40-100mW, and an emission filter of 400nm center wavelength with a 40nm bandwidth. SHG images of the entire depth of the normal murine colon were used to precisely quantify the area, circularity, volume, depth, of crypts, and the collagen density and orientation of normal crypts, and compared them to crypts at varying time points in dysplastic progression. In healthy, 18 week old A/J mice, the algorithm has calculated an average crypt size of 700 square microns, average circularity of 0.83, and approximately 560 crypts per square millimeter of epithelium.

10070-55, Session 12

Improving resolution and SNR in saturated excitation microscopy by using signal subtraction

Yasunori Nawa, Yasuo Yonemaru, Osaka Univ. (Japan); Atsushi Doi, Olympus Corp. (Japan); Atsushi Kasai, Nicholas I. Smith, Hitoshi Hashimoto, Satoshi Kawata, Katsumasa Fujita, Osaka Univ. (Japan)

The recent development in super-resolution microscopy has been expanding their applications to various types of biological samples. As one of the super-resolution microscope technique that can improve the spatial resolution in 3D imaging, we have proposed the use of saturation of fluorescence excitation in confocal microscopy. The highly nonlinear fluorescence responses induced by the saturated excitation (SAX) is localized within a focal volume and, therefore, can contribute to reconstructing fluorescence images with the spatial resolution beyond the diffraction limit. By using SAX microscopy, high-resolution 3D imaging of 3D-cultured cells has demonstrated.

Here, we present a technique to increase the signal to noise ratio (SNR) of SAX microscopy that provides the improvement of spatial resolution and image contrast of finer structures of a sample. In this technique, a fluorescence sample is scanned two or three times with different excitation intensities. Each excitation intensity is set to induce different levels of saturation in fluorescence signals so that the signal components nonlinearly proportional to the excitation intensity can be extracted by comparison of the fluorescence intensities. Compared to the harmonic demodulation technique presented in our past reports, the signal amount is improved 8 and 32 times in detection of 2nd and 3rd order of nonlinear fluorescence signals. We confirmed the improvement of the spatial resolution and the image contrast with the subtraction technique in the observations of fluorescent beads samples and a fluorescent-stained mouse brain tissue. We also confirmed that the subtraction technique can be used to improve the spatial resolution of two-photon excitation microscopy.

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10071-1, Session 1

Quantitative fluorescence correlation spectroscopy on DNA in living cells

Rudra P. Kafle, Cameron Hodges, Jens-Christian D. Meiners, Univ. of Michigan (United States)

FCS is a fluorescence technique conventionally used to study the kinetics of fluorescent molecules in a dilute solution. Being a non-invasive technique, it is now drawing increasing interest for the study of more complex systems like the dynamics of DNA or proteins in living cells. Unlike an ordinary dye solution, the dynamics of macromolecules like proteins or entangled DNA in crowded environments is often slow and subdiffusive in nature. This in turn leads to longer residence times of the attached fluorophores in the excitation volume of the microscope and artifacts from photobleaching abound that can easily obscure the signature of the molecular dynamics of interest and make quantitative analysis challenging.

We discuss methods and procedures to make FCS applicable to quantitative studies of the dynamics of DNA in live prokaryotic and eukaryotic cells. The intensity autocorrelation is computed function from weighted arrival times of the photons on the detector that maximizes the information content while simultaneously correcting for the effect of photobleaching to yield an autocorrelation function that reflects only the underlying dynamics of the sample. This autocorrelation function in turn is used to calculate the mean square displacement of the fluorophores attached to DNA. The displacement data is more amenable to further quantitative analysis than the raw correlation functions. By using a suitable integral transform of the mean square displacement, we can then determine the viscoelastic moduli of the DNA in its cellular environment. The entire analysis procedure is extensively calibrated and validated using model systems and computational simulations.

10071-2, Session 1

Time resolved STED and HOMO FRET dynamics in EGFP

Angus J. Bain, Univ. College London (United Kingdom); Thomas A. Masters, Univ. of Cambridge (United Kingdom); Richard J. Marsh, King's College London (United Kingdom); Thomas S. Blacker, Daven A. Armoogum, Univ. College London (United Kingdom); Banafshe Larijani, Unidad de Biofísica (Spain)

Stimulated emission depletion (STED) allows the circumvention of electric dipole selection rules which restrict the sensitivity of fluorescence observables to the second order (rank K=2) transition dipole moment correlation function. Here we combine time resolved fluorescence anisotropy with polarisation resolved STED measurements to determine the evolution of the degrees of quadrupolar (K=2) and hexadecapolar (K=4) transition dipole moment alignment in EGFP following two-photon excitation at 800nm.

Both rotational diffusion and HOMO-FRET are found to contribute to excited state depolarisation in EGFP. HOMO FRET relaxation of the hexadecapole transition dipole alignment is found to be more rapid than that observed in conventional fluorescence anisotropy (K=2) measurements.These results indicate that the measurement of higher order (K>2) transition dipole moment correlation functions are likely to prove a more sensitive probe of resonance energy transfer dynamics.

10071-3, Session 1

Extracting the average single-molecule biexciton photoluminescence lifetime from a solution of chromophores

Thomas S. Bischof, Justin R. Caram, Andrew P. Beyler, Moungi G. Bawendi, Massachusetts Institute of Technology (United States)

In a fluorescence correlation spectroscopy (FCS) experiment, we can measure the average behavior of individual molecules, averaged over freely-diffusing emitters in solution. In our prior work we used this single-molecule contrast to study the biexciton quantum yield of colloidal quantum dots, and here we describe an extension which permits direct measurement of the average biexciton lifetime. This method deconstructs photon correlation events arising from a single pulse of excitation, which may be divided into events associated with the emission by a biexciton and the subsequent monoexciton. After accounting for the Poissonian background, we are able to resolve the true biexciton dynamics, as well as those of the monoexciton.

10071-5, Session 2

Single-molecule metal induced energy transfer

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We present a new concept for measuring distance values of single molecules from a surface with nanometer accuracy using the energy transfer from the excited molecule to surface plasmons of a metal film [1]. We measure the fluorescence lifetime of individual dye molecules deposited on a dielectric spacer as a function of a spacer thickness. By using our theoretical model [2], we convert the lifetime values into the axial distance of individual molecules. Similar to Förster resonance energy transfer (FRET), this allows emitters to be localized with nanometer accuracy, but in contrast to FRET the distance range at which efficient energy transfer takes place is an order of magnitude larger. Together with orientation measurements [3], one can potentially use smMIET to localize single emitters with a nanometer precision isotropically, which will facilitate intra- and intermolecular distance measurements in biomolecules and complexes, circumventing the requirement of the knowledge of mutual orientations between two dipole emitters which severely limits the quantification of such distances from a conventional single-pair FRET (spFRET) experiment.

[1] Karedla, N., Chizhik, A.I., Gregor, I., Chizhik, A.M., Schulz, O., Enderlein, J., ChemPhysChem, 15, 705-711 (2014).

[2] Enderlein J., Biophyical Journal, 78, 2151-8 (2000).

[3] Karedla, N., Stein, S. C., Hähnel, D., Gregor, I., Chizhik, A., & Enderlein, J., Physical Review Letters, 115, 173002 (2015).

10071-6, Session 2

Spectroscopic photon localization microscopy: breaking the resolution limit of single molecule localization microscopy

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Distinguishing minute differences in spectroscopic signatures is crucial for revealing the fluorescence heterogeneity among fluorophores to achieve a high molecular specificity. Here we report spectroscopic photon localization microscopy (SPLM), a newly developed far-field spectroscopic imaging technique, to achieve nanoscopic resolution based on the principle of single-molecule localization microscopy while simultaneously uncovering the inherent molecular spectroscopic information associated with each stochastic event (Dong et al., Nature Communications 2016, in press). In SPLM, by using a slit-less monochromator, both the zero-order and the first-order diffractions from a grating were recorded simultaneously by an electron multiplying charge-coupled device to reveal the spatial distribution and the associated emission spectra of individual stochastic radiation events, respectively. As a result, the origins of photon emissions from different molecules can be identified according to their spectral differences with sub-nm spectral resolution, even when the molecules are within close proximity. With the newly developed algorithms including background subtraction and spectral overlap unmixing, we established and tested a method which can significantly extend the fundamental spatial resolution limit of single molecule localization microscopy by molecular discrimination through spectral regression. Taking advantage of this unique capability, we demonstrated improvement in spatial resolution of PALM/STORM up to ten fold with selected fluorophores. This technique can be readily adopted by other research groups to greatly enhance the optical resolution of single molecule localization microscopy without the need to modify their existing staining methods and protocols. This new resolving capability can potentially provide new insights into biological phenomena and enable significant research progress to be made in the life sciences.

10071-7, Session 2

Maximum likelihood estimation of pupil functions in 3D single-molecule localization microscopy

Petar N. Petrov, Yoav Shechtman, William E. Moerner, Stanford Univ. (United States)

Point spread function (PSF) engineering has extended far-field localization microscopy into three dimensions by encoding the axial position of each emitter into the shape of its image on the detector. By fitting the observed PSF to a model function, one can extract position information with sub-diffraction precision. However, in practice this procedure is often complicated by optical aberrations present in the imaging system, which distort the shape of the observed PSF relative to the model function. The mismatch between the model and observed PSFs can limit the accuracy and precision achieved by the localization procedure.

Here, we present a simple method to experimentally improve the model PSF by phase retrieval of the pupil function of the imaging system using a set of images of an isolated emitter at different displacements from the focal plane. The pupil function is estimated by adding a phase term consisting of a combination of Zernike modes to the theoretical electric field at the back focal plane of the microscope. The amplitudes of the Zernike modes are determined by maximizing the likelihood function over all pixels in the experimental data set. Importantly, since all data is taken with the phase mask in place, we account for any aberrations it introduces. Using the resulting pupil function, we generate a model PSF which is significantly improved over the theoretical model in both the accuracy and precision of experimental emitter localizations. We also provide a MATLAB package which performs the entire fitting procedure, from phase retrieval to single-emitter localization.

10071-8, Session 2

Nanoscopic imaging of chromatin topology utilizing intrinsic fluorescence from unmodified nucleic acids

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Imaging the nanoscale intracellular structures formed by nucleic acids, such as chromatin, in non-perturbed, structurally and dynamically complex cellular systems, will help improve our understanding of biological processes and open the next frontier for biological discovery. Current optical super-resolution fluorescence techniques require exogenous labels that may disrupt cell function and alter the subdiffractional macromolecular structures they are used to visualize. As a means for label-free optical super-resolution imaging, we examined the discovery of stochastic fluorescence switching of unmodified nucleic acids under visible light illumination. Utilizing this phenomenon and a single-molecule photon localization approach we generated subdiffraction-resolution images down to ~20nm using intrinsic fluorescence from nucleic acids. Specifically, the nanoscale organization of interphase nuclei and mitotic chromosomes were imaged. Using such a method for visualization, we performed a quantitative analysis of the DNA occupancy level and a subdiffractional analysis of the chromosomal organization. These experiments demonstrate a new method for visualizing the nanoscopic features of macromolecular structures composed of nucleic acids without the need for exogenous labels.

10071-9, Session 3

Bundled microtubules structural arrangement dynamics monitored in real time using multiple-pulse pumping super resolution technology

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We present a new application of multi-pulse pumping technology with time-gated detection (MPP-TGD) to increase image resolution and significantly improve image collection speed. Switching from single pulse excitation to multiple-pulse excitation within one excitation trace instantaneously increases the fluorescence intensity of a microtubule labeled with a long-lived fluorescent probe allowing for its quick localization. This on-demand intensity change is done at MHz frequencies allowing for high-speed system localization. With this approach, we obtain a higher fluorescence signal compared to single molecule stochastic approaches allowing high-speed super-resolved image collection, opening a new way to observe real-time functional information and probing of structural rearrangements at the nanometer scale. We use this technique to study long microtubules labeled with multiple antibodies (minibodies) that carry a significant number of dye molecules. Using MPP-TGD, we can quickly test changes in microtubule bundle organization induced by injury. This method may have a broad impact on our understanding of many neurodegenerative diseases.

10071-10, Session 3

Quantum enhanced superresolution microscopy

Dan Oron, Ron Tenne, Yonatan Israel, Yaron Silberberg, Weizmann Institute of Science (Israel)

Far-field optical microscopy beyond the Abbe diffraction limit, making use of nonlinear excitation (e.g. STED), or temporal fluctuations in fluorescence (PALM, STORM, SOFI) is already a reality. In contrast, overcoming the diffraction limit using non-classical properties of light is very difficult to achieve due to the fragility of quantum states of light. Here, we experimentally demonstrate superresolution microscopy based on quantum properties of light naturally emitted by fluorophores used as markers in fluorescence microscopy. Our approach is based on photon antibunching, the tendency of fluorophores to emit photons one by one rather than in bursts. Although a distinctively quantum phenomenon, antibunching is readily observed in most common fluorophores even at room temperature.

This nonclassical resource can be utilized directly to enhance the imaging resolution, since the non-classical far-field intensity correlations induced by antibunching carry high spatial frequency information on the spatial distribution of emitters. Detecting photon statistics simultaneously in the entire field of view, we were able to detect non-classical correlations of the second and third order, and reconstructed images with resolution significantly beyond the diffraction limit.

Alternatively, we demonstrate the utilization of antibunching for augmenting the capabilities of localization-based superresolution imaging in the presence of multiple emitters, using a novel detector comprised of an array of single photon detectors connected to a densely packed fiber bundle. These features allow us to enhance the spatial and temporal resolution with which multiple emitters can be imaged compared with other techniques that rely on CCD cameras.

10071-11, Session 3

Non-linear image scanning microscopy

Ingo Gregor, Georg-August-Univ. Göttingen (Germany); Robert Ros, Arizona State Univ. (United States); Jörg Enderlein, Georg-August-Univ. Göttingen (Germany)

Nowadays, multiphoton microscopy can be considered as a routine method for the observation of living cells, organs, up to whole organisms. Second-harmonics generation (SHG) imaging has evolved to a powerful qualitative and label-free method for studying fibrillar structures, like collagen networks. However, examples of super-resolution non-linear microscopy are rare. So far, such approaches require complex setups and advanced synchronization of scanning elements limiting the image acquisition rates.

We describe theory and realization of a super-resolution image scanning microscope [1, 2] using two-photon excited fluorescence as well as second-harmonic generation. It requires only minor modifications compared to a classical two-photon laser-scanning microscope and allows image acquisition at the high frame rates of a resonant galvo-scanner. We achieve excellent sensitivity and high frame-rate in combination with two-times improved lateral resolution. We applied this method to fixed cells, collagen hydrogels, as well as living fly embryos. Further, we proofed the excellent image quality of our setup for deep tissue imaging.

1. Müller C.B. and Enderlein J. (2010) Image scanning microscopy. Phys. Rev. Lett. 104(19), 198101.

2. Sheppard C.J.R. (1988) Super-resolution in confocal imaging. Optik (Stuttg) 80 53–54.

10071-12, Session 3

Image scanning microscopy using a SPAD detector array

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The use of an array of detectors can help overcoming the traditional limitation of confocal microscopy: the compromise between signal and theoretical resolution.

Each element independently records a view of the sample and the final image can be reconstructed by pixel reassignment or by inverse filtering (e.g. deconvolution).

In this work, we used a SPAD array of 25 detectors specifically designed for this goal and our scanning microscopy control system (Carma) to acquire the partial images and to perform online image processing.

Further work will be devoted to optimize the image reconstruction step and to improve the fill-factor of the detector.

10071-13, Session 4

Combining PALM and SOFI for quantitative imaging of focal adhesions in living cells

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Focal adhesions are complicated assemblies of hundreds of proteins that allow cells to sense their extracellular matrix and to adhere to it. Although most focal adhesion proteins have been identified, their spatial organization in living cells remains challenging to observe. Photo-activated localization microscopy (PALM) is an interesting technique for this purpose, especially since it allows estimation of molecular parameters such as the fluorophore density. However, focal adhesions are dynamic entities, requiring a temporal resolution well below one minute, which is difficult to achieve by PALM.

In order to address this problem, we merged PALM with super-resolution optical fluctuation imaging (SOFI) by applying both techniques to the same data. Since SOFI tolerates an overlap of single molecule images, it can improve the temporal resolution compared to PALM. Moreover, an adaptation called balanced SOFI (bSOFI) allows estimation of molecular parameters as well. We therefore performed simulations in order to assess the complementarity between PALM and SOFI for quantitative imaging. We demonstrated the potential of our PALM–SOFI concept as a quantitative imaging framework by investigating moving focal adhesions in living cells.

10071-14, Session 4

Superresolution upgrade for confocal spinning disk systems using image scanning microscopy

Sebastian Isbaner, Dirk Hähnel, Ingo Gregor, Jörg Enderlein, Georg-August-Univ. Göttingen (Germany)

Confocal Spinning Disk Systems are widely used for 3D cell imaging because they offer the advantage of optical sectioning at high framerates and are easy to use. However, as in confocal microscopy, the imaging resolution is diffraction limited, which can be theoretically improved by a factor of 2 using the principle of Image Scanning Microscopy (ISM) [1]. ISM with a Confocal Spinning Disk setup (CSDISM) has been shown to improve contrast as well as lateral resolution (FWHM) from 201 ± 20 nm to 130 ± 10 nm at 488 nm excitation. A minimum total acquisition time of one second per ISM image makes this method highly suitable for 3D live cell imaging [2]. Here, we present a multicolor implementation of CSDISM for the popular Micro-Manager Open Source Microscopy platform. Since changes in the optical path are not necessary, this will allow any researcher to easily upgrade their standard Confocal Spinning Disk system at remarkable low cost (~5000 USD) with an ISM superresolution option.

[1]. Müller, C.B. & Enderlein, J. Image Scanning Microscopy. Physical Review Letters 104, (2010).

[2]. Schulz, O. et al. Resolution doubling in fluorescence microscopy with confocal spinning-disk image scanning microscopy. Proceedings of the National Academy of Sciences of the United States of America 110, 21000-5 (2013).

10071-15, Session 4

Resolution enhancement down to 10-nm based on saturated excitation (SAX) microscopy plus novel nonlinear response

Hou-Xian Ding, Kuan-Yu Li, Gitanjal Deka, I-Cheng Su, Shi-Wei Chu, National Taiwan Univ. (Taiwan)

Superresolution microscopies have revolutionized optical imaging field in the last decade by providing a novel capability for nanoscale observation with visible light. Current techniques mostly rely on switching or saturation of fluorescence, but suffer from limited imaging depth due to the requirement of special illumination patterns (STED, SIM), or the lack of optical sectioning capability (localization microscopy). Saturated excitation (SAX) microscopy provides the potential for deep-tissue resolution enhancement due to its laser-scanning nature without additional beam shape engineering. However, for current fluorescence SAX microscopy, it is difficult to achieve resolution better than 100-nm, limited by the difficulty to obtain high order demodulation as well as by photobleaching due to high-intensity illumination.

Our recent finding revealed that the bleaching issue in SAX could be resolved by substituting fluorescence with scattering from metallic nanoparticles. From the scattering-based experiment, we realized that the resolution limit of SAX could be significantly improved by proper nonlinear response of emitters. In this paper, we show that with suitable nonlinear power dependence, either scattering or fluorescence, SAX microscopy can provide sub-20-nm spatial resolution at relatively low power. Our work provides not only a new concept to enhance resolution with saturation-based techniques, but also a novel example toward ultrahigh resolution imaging with a laser-scanning scheme.

10071-16, Session 5

Light harvesting plasmonic quantum dots with regulated optical output *(Invited Paper)*

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Plamonic hybrids incorporating metal nanoparticles and semiconducting materials like conjugated polymers and colloidal quantum dots have been proposed for biosensing and optoelectronic applications. The ability to control the plasmon-exciton interaction in such materials is crucial for the resulting properties of the plasmonic hybrid. In this presentation we demonstrate control of the optical (photoluminescence) output of plasmonic hybrids by the use of DNA self-assembly in combination with optically inactive molecular spacers [1.2] We demonstrate tunability of the optical signal of a given hybrid, from efficient quenching to efficient enhancement of the emitted photoluminescence. We propose the use of alternating laser excitation single particle spectroscopy as a straightforward method for characterizing the plasmon-exciton interaction in such hybrids and assessing the enhancement in photoluminescence from isolated plasmonic hybrids.

1. M.M.Maye, O.Gang, M.Cotlet, Photoluminescence enhancement in single Qdot-Au nanoparticle DNA-linked heterodimers probed by single molecule spectroscopy, Chem Comm. 2010 (46) 6111.

D.Sun, Y.Tian, Y.Zhang, Z.Xu, M.Y.Sfeir, M.Cotlet*, and O.Gang*, Light Harvesting Nanoparticle Core-Shell Clusters with Controllable Optical Output, ACS Nano 2015, 9, 5657.

10071-17, Session 5

Correlated fluorescence-atomic force microscopy studies of the clathrin mediated endocytosis in SKMEL cells

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Clathrin-mediated endocytosis is one of the central pathways for cargo transport into cells, and plays a major role in the maintenance of cellular functions, such as intercellular signaling, nutrient intake, and turnover of plasma membrane in cells. The clathrin-mediated endocytosis process involves invagination and formation of clathrin-coated vesicles. However, the biophysical mechanisms of vesicle formation are still debated. Currently, there are two competing models describing the membrane bending during the formation of clathrin cages: the first involves the deposition of all clathrin molecules to the plasma membrane, forming a flat lattice prior to membrane bending to form clathrin vesicles, whereas in the second model, membrane bending happens simultaneously as the clathrin arrives to the site to form a clathrin-coated cage. We investigate clathrin vesicle formation mechanisms through the utilization of tapping-mode atomic force microscopy for high resolution topographical imaging in neutral buffer solution of unroofed cells exposing the inner membrane, combined with fluorescence imaging to definitively label intracellular constituents with specific fluorescent fusion proteins (actin filaments labeled with green phalloidin-antibody and clathrin coated vesicles with the fusion protein Tq2) in SKMEL (Human Melanoma) cells. Results from our work are compared against dynamical polarized total internal fluorescence (TIRF), super-resolution photo-activated localization microscopy (PALM) and transmission electron microscopy (TEM) data to draw conclusions regarding the prominent model of vesicle formation in clathrin-mediated endocytosis.

10071-18, Session 5

Improved timing and diffusivity measurement in single-molecule recycling in a nanochannel

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Single-molecule recycling (SMR) in a nanochannel, in which a molecule in solution quickly passes through a focused laser beam and the solution flow is reversed after a set delay following each passage, provides an attractive alternative to feedback-driven trapping for prolonging the observation of

a single molecule in a confocal microscope, as the molecule is periodically and most of the time in the dark, which extends the time before irreversible photobleaching and also gives time for recovery from photogenerated reversible dark states between passages. Guided by suggestions in previous SMR reports, we have utilized a National Instruments FPGA card and LabVIEW Realtime to implement 10 ns photon time-stamping, weighted sliding sum digital filtering, maximum-likelihood (ML) analysis of photon time-stamps, and real-time control of electrokinetic voltage in SMR experiments in order to improve the detection and timing of passages of the single molecule through the focused laser spot. We have developed a ML technique for measuring the diffusivity of the single molecule in the nanochannel, which uses a look-up table to update the probability density function of the diffusivity with each detected passage, thereby also providing confidence limits for the measurement. We use Monte Carlo simulations to examine prior experiments, validate the ML diffusivity measurement strategy, and evaluate choice of experimental parameters.

10071-19, Session 6

Molecular counting of membrane receptor subunits with single-molecule localization microscopy *(Invited Paper)*

Carmen Krüger, Franziska Fricke, Christos Karathanasis, Marina S Dietz, Sebastian Malkusch, Johann Wolfgang Goethe-Univ. Frankfurt am Main (Germany); Gerhard Hummer, Max Planck Institute of Biophysics, Department of Theoretical Biophysics (Germany); Mike Heilemann, Johann Wolfgang Goethe-Univ. Frankfurt am Main (Germany)

Knowledge of assembly and subunit architecture of macromolecular complexes in a cellular context is essential to infer their biological function. Fluorescence microscopy has become increasingly popular for quantifying molecular numbers in the cellular environment [1]. However at high protein densities, the spatial resolution limit of 200 nm in conventional microscopy hampers direct observation of single protein complexes. Super-resolution fluorescence techniques present a powerful solution to bypass this limit. Single-molecule localization microscopy (SMLM) is particularly well suited, as next to high-resolution images of cellular structures, it potentially provides quantitative information on the detection of single emitters.

The analysis of fluorophore blinking events in an SMLM experiment offers a promising route to probe oligomeric states in protein complexes [2-5]. We demonstrate the practical applicability of this approach by quantifying the oligomerization states of several membrane proteins tagged with the mEos2 fluorescent protein. We further applied this method to investigate mixed populations of membrane receptors with different stoichiometry, as they are found for toll-like receptors and death receptors. This method should be robust and broadly applicable to counting co-localized molecules in vivo and in vitro.

[1] F. Fricke et al., ChemPhysChem 16, 713 (2015)

[2] S.-H. Lee et al., Proc Natl Acad Sci 109, 17436-17441 (2012)

[3] S. Avilov et al., Plos One 9(6):e98362 (2014)

[4] F. Fricke et al., Sci Rep 5, 14072 (2015)

[5] G. Hummer et al., Mol Biol Cell (2016)

10071-20, Session 6

Single cell genomic quantification by non-fluorescence nonlinear microscopy

Jing Liu, South Dakota School of Mines and Technology (United States)

Fluorescence-based single molecule techniques to interrogate gene expression present a very low signal-to-noise ratio due to the strong autofluorescence and other background signals from biological components. In this presentation, we present two non-fluorescent and background-free imaging methods to quantify the mRNA of Human epidermal growth receptor 2 (Her2) at single molecule resolution in single cells. First of all, gold nanoparticles (AuNPs) are utilized as an orthogonal probe for non-fluorescence detection of single molecules with a transient absorption microscopy (TAM), which allows background-free quantifications of single mRNA transcript and label-free imaging of biological components (background). Secondly, coherent second harmonic generation (SHG) emission from individual barium titanium oxide (BTO) nano-probes was demonstrated, allowing for stable signal beyond the autofluorescence window. Her2 surface marker and Her2 mRNA were specifically labeled with BTO probes, and Her2 mRNA was quantified at single copy sensitivity in Her2 expressing phenotypes directly in cancer tissues. Furthermore, a non-fluorescent super resolution imaging method based on the SHG was developed to quantify individual Her2 mRNAs in single cells. Our demonstration shows that these non-fluorescent imaging approaches have the potential to provide new dimensions in biomarker quantification at single molecule sensitivity in turbid biological environments, offering a strong cross-platform strategy for clinical monitoring at single cell resolution in situ.

10071-21, Session 6

Multiple single-molecule nano-tracking based on spectral discrimination of fluorescent probes

Taro Ichimura, Taishi Kakizuka, Junichi Kaneshiro, Tomonobu Watanabe, RIKEN (Japan)

Single-molecule measurement techniques have advanced tremendously over the past two decades, and have helped deepen the understanding of the working mechanisms of linear motor proteins. To further understand the protein functioning, attention is now shifting upward through the hierarchy, from individual elements to the cooperative behavior of the supramolecular system, suggesting the need of an additional technique that transcends conventional single-molecule measurement. In this study, we developed a fluorescence microscope system for simultaneous observation of dynamic behaviors of multiple proteins within a sub-diffraction-limit scale [1]. The measurement scheme is based on combination of multicolor fluorescent labeling of target proteins and imaging spectroscopy. A wide-field fluorescence image is diffracted by a grating to separate distinct colors and detected by an EM-CCD camera detector. Using quantum dot probes of distinct colors, we experimentally verified the localization precision to be a few nanometers at temporal resolution of 30 ms or faster. One-dimensional processive movement of two heads of a single myosin molecule (myosin V) along an actin filament was successfully traced with clear visualization of the hand-over-hand manner of the myosin's walk. We also succeeded in simultaneous tracking of four myosin molecules, which showed that the four single proteins were chasing each other back and forth within sub-diffraction-limit scale. Furthermore, the optical system was modified for two-dimensional measurement, and applied to two-dimensional tracking of multiple myosin molecules [1,2]. Our approach is useful for investigating cooperative movement of proteins in supramolecular nanomachinery.

[1] Kakizuka et al., Opt. Express 7, 2475 (2016).

[2] Ichimura, Japan Patent, P2015-22070A (2015).

10071-22, Session 6

Nanoscale imaging of solid state particles for bio applications

Denitza Denkova, Martin Ploschner, Alf Garcia-Bennett, Lindsay M. Parker, Yiqing Lu, James A. Piper, Macquarie Univ. (Australia)

A novel class of solid state biological markers, such as upconversion nanoparticles (UCNPs), nanoruby, nanodiamond, has been extensively explored last years as a promising alternative to molecular dyes. These nanoparticles have the immense advantage of being non-photobleaching. Additionally, UCNPs have long tunable lifetimes which allow for multiplexing and for eliminating autofluorescence background in bio-imaging.

Unlike molecular dyes, nanoparticles do not stain a full volume of bio-matter, but instead allow a finite number of nanoparticles conjugated to the target of interest – for example a cell receptor. The advantage of this is that it allows one to target, quantify, track and visualize individual nanostructures in cells over a long time period.

Respectively, it is crucial to develop and apply techniques able to visualize and analyse individual nanoparticles in biological environment. This is a challenging task, as the long-lifetime nanoparticles typically have low emission efficiency and are difficult to image on an individual level. We suggest different approaches to access this information with resolution and sensitivity down to single nanoparticles. We are exploring the possibility to optically observe nanoparticles with sub-diffraction resolution. A co-localized optical and atomic force microscopy platform provides access to additional structural and chemical information. Initial experiments suggest that both techniques allow accessing single nanoparticles and obtaining information about their bio-conjugation and behaviour in biological environment with resolution down to 100 nm.

Thus, the proposed methods to measure nanoparticles with sub-100nm resolution in biological environment are a crucial step towards the successful application of nanoparticles as bio-markers.

10071-23, Session 7

Fast TCSPC based confocal microscopy optimised for Hz image frame rates with high photon throughput *(Invited Paper)*

Benedikt Kraemer, Paja Reisch, Marcus Sackrow, Sandra Orthaus-Mueller, Sebastian Tannert, Felix Koberling, PicoQuant GmbH (Germany); Piau Siong Tan, Edward Lemke, EMBL Heidelberg (Germany); Rainer Erdmann, PicoQuant GmbH (Germany)

Fluorescence lifetime imaging (FLIM) as a tool for monitoring fast, dynamic processes is becoming more important in fields such as live cell imaging. New imaging approaches like rapidFLIM are increasingly demanding in terms of data throughput rates and short frame acquisition times. In order to meet these requirements, we combined fast confocal imaging capabilities including STED super-resolution with a superior single photon counting throughput concept in a single system, while maintaining key features needed for single molecule sensitivity.

We expanded the MicroTime 200 time-resolved microscopy platform with a fast 3 mirror galvo scanner allowing both fast imaging with up to several FLIM frames per second and stable point positioning using the same beam path and within an experimental sequence. Furthermore, by exploiting recent hardware developments such as TCSPC modules with ultra short dead times and hybrid photomultiplier detector assemblies, significantly higher detection count rates (> 10 Mcps) can be used for imaging while maintaining good temporal accuracy.

We took special care to ensure synchronisation between pixel dwell time and laser repetition rate. This enables generation of an equal number of laser pulses per pixel, which is especially important for fast imaging with high pixel numbers per line and e.g., phosphorescence imaging requiring low laser repetion rates.

In this presentation, we will demonstrate the speed, accuracy, and versatility of our prototype by means of multispecies STED imaging as well as single molecule detection.

10071-24, Session 7

Different nano-environment of cytochrome C oxidase and FoF1-ATP synthase in mitochondrial cristae of living human cells by FLIM and superresolution microscopy

Franziska Förtsch, Ralf Mrowka, Christoph U. Biskup, Michael Börsch, Universitätsklinikum Jena (Germany)

Cytochrome C oxidase and FoF1-ATP synthase constitute complex IV and V, respectively, of the five membrane-bound enzymes in mitochondria comprising the respiratory chain. These enzymes are located in the inner mitochondrial membrane (IMM), which exhibits large invaginations called cristae. According to recent cryo-tomography, FoF1-ATP synthases are located predominantly at the rim of the cristae, while cytochrome C oxidases are likely distributed in planar membrane areas of the cristae. Previous FLIM measurements (K. Busch and coworkers, BBA-Bioenergetics 2016) of complex II and III unravelled differences in the local environment of the membrane enzymes in the cristae. Here, we tagged complex IV and V with mNeonGreen and investigated their mitochondrial nano-environment by FLIM and superresolution microscopy in living human cells. Different lifetimes were found and will be discussed in terms of Förster resonance energy transfer and cristae morphology and plasticity.

10071-25, Session 7

16ch time-resolved single-molecule spectroscopy using line excitation

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Single-molecule spectroscopy based on freely-diffusing or flowing molecules allows detecting conformational changes of biomolecules without interferences from surface immobilization. In addition to intensity-based analysis, the ability to resolve lifetimes of observed species increases the sensitivity in detecting local environment or conformational changes and overcomes artifacts common in intensity-based measurements.

Common to all freely-diffusing techniques, however, are the long acquisition times which limit the ability to track kinetics in realtime and render the exploration of large space of experimental conditions cost prohibitive.

This limitation can be addressed by multiplexing both excitation and detection in so called multispot experiments. However, so far, only intensity-based multispot experiments have been reported mainly because of the lack of multi-channel TCSPC hardware to be coupled to the detectors arrays.

Here we report a demonstration of the first time-resolved multispot system employing a novel 16-channels SPAD array and a 16-channels TCSPC acquisition hardware.

The multispot excitation is achieved by shaping a 532nm pulsed laser into a line which matches the linear arrangement 16 SPAD pixels.

This excitation scheme greatly simplifies the optical setup and alignment procedure while achieving single-molecule sensitivity and low background levels, comparable to a previously demonstrated SLM-based approach.

We demonstrate that the line-excitation technique is a robust and cost-effective approach to implement multispot systems (both intensity- or lifetime-based) employing linear detector arrays.

10071-26, Session 8

Super-resolution localization microscopy of unstained nanostructures

Ben E. Urban Jr., Biqin Dong, The-Quyen Nguyen, Vadim Backman, Cheng Sun, Hao F. Zhang, Northwestern Univ. (United States)

Conventional optical imaging cannot provide the spatial resolutions necessary for many nanoscopic studies. The rapid progress of super-resolution optical imaging has created the possibility for many novel investigations of nanoscopic structures. However, the majority of super-resolution techniques rely on extrinsic contrast agents. Extrinsic agents have several weaknesses, including (1) they require additional labeling processes; (2) they may modify the physical properties of the target material; and (3) they could introduce inaccurate spatial localization caused by the physical dimension of the tagged fluorescent and linker molecules. The combination of these weaknesses reduces the appeal of extrinsic fluorescent contrast agents. In fact, topological and chemical defects in polymeric molecules can result in various photophysical interactions, including energy transfer, ground- or excited-state aggregate formation, and charge transfer. These photophysical processes can significantly modify some of the polymer's optical properties that may be suitable for optical super-resolution imaging. Here we describe a method for nanoscopic optical imaging of buried polymer nanostructures without the need for extrinsic staining. We observed intrinsic stochastic fluorescence emission or blinking from unstained polymers and performed spatial-temporal spectral analysis to investigate its origin. We further applied photon localization super-resolution imaging reconstruction to the detected stochastic blinking, and achieved a spatial resolution of up to 60 nm, which corresponds to a six-fold increase over the optical diffraction limit. This work demonstrates the potential for studying the intrinsic molecular-specific properties at sub-diffraction-limited optical resolutions.

10071-27, Session 8

Structured light illuminator for on-chip stimulated emission depletion microscopy

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Diffraction-limited optical microscopy systems have traditionally hindered our ability to visualise and study biological cells and sub-cellular structures. Newer super-resolution fluorescence microscopy techniques like stimulated emission depletion (STED) [1] microscopy have overcome this diffraction barrier by stimulating emission from the periphery of the focus of a sample, thereby depleting all excited fluorophores in that region and limiting spontaneous fluorescence emission to a small area in the centre of the sample. This is achieved by employing a secondary dough-nut shaped laser beam of a slightly lower energy compared to the excitation energy of the fluorophore. The excited fluorophore absorbs a photon of this secondary wavelength and relaxes to the ground state. The emission wavelength of this stimulated relaxation corresponds to the energy of the STED photon. This response is filtered out from the detector.

On-chip beam structuring involving integrated photonic devices can drastically simplify current complicated STED setups which are built to generate the structured beam and scan large fields of view. Designing these devices on silicon-on-insulator (SOI) is infeasible since silicon is known to be absorptive at wavelengths below 1.1μm where fluorophore excitation and emission wavelengths lie. Silicon nitride films, however, are transparent to wavelengths in the visible range (532 nm) and also enable the fabrication of photonic structures on top of standard complementary metal-oxide semiconductor (CMOS) electronics [2].

We designed focusing grating couplers[3] with finite element method simulations and arranged them in mirrored pairs, with feeds that split from a multimode interference coupler, such that their focus spots perfectly overlap and the signals were perfectly out of phase with each other. We simulated configurations with multiple grating couplers to generate focussed dough-nut shaped spots with hollow cores. Measurements demonstrated the performance of our devices.

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10071-28, Session 8

3D superresolution imaging with stimulated emission depletion SIM

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Structured illumination microscopy (SIM) achieves superresolution by using illumination or depletion pattern during wide-field fluorescence imaging. Information beyond the diffraction limit is embedded in the Moiré pattern and retrieved through Fourier space reconstruction. 3D linear SIM can improve both lateral and axial resolution by 2 fold. Nonlinear SIM techniques, including Saturated SIM and Photo-switch SIM, combine nonlinear effect with structured illumination to obtain higher than 2-fold resolution enhancement in lateral dimensions, but little attention was paid to the axial resolution of nonlinear SIM, which was assumed to be still at the diffraction limit.

In principle, all nonlinear optical effect can be applied to nonlinear SIM. Stimulated Emission Depletion (STED) was long been proposed as a candidate. We report a new nonlinear SIM approach that utilizes the STED effect to achieve superresolution. STED-SIM offers a unique combination of fast imaging speed, large field of view, and 3D resolution beyond the diffraction limit.

We demonstrate that STED-SIM reaches a 55 nm resolution at far-red wavelength range, which is roughly 5 times of the diffraction limit. The acquisition time needed for such 5-fold superresolution image is 7-8 seconds. We further demonstrate that when choosing a proper nonlinear effect in SIM, resolution enhancement along the axial dimension can be achieved. In the case of STED-SIM, a low coherent STED field caused strong axial confinement on the structured STED effect. Due to the nonlinearity of STED effect, the contrast of the structured STED effect quickly deteriorates with defocus. This local confinement of structured nonlinear effect provides strong optical sectioning and therefore brings axial resolution improvement. 3D cellular imaging at 100 nm slice thickness has been achieved.

10071-29, Session PSun

Expanding the capabilities of single molecule STED with advanced pulsed interleaved excitation

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Stimulated Emission Depletion (STED) microscopy has evolved into an

established imaging method offering super-resolution well beyond 50 nm. Whereas STED is now available in many laboratories, it is still in the focus of research to push the boundaries of its capabilities and applications. Time-resolved STED microscopy using time correlated single photon counting (TCSPC) is advantageous for many applications and promises further development for increased resolution and less photo-damage.

Here, we show the application of established methods (e.g., gSTED) as well as emerging applications of time-resolved STED. We employ pulsed interleaved excitation (PIE), where the STED laser is pulsed at half the frequency of the excitation laser, such that STED and confocal data is taken practically at the same time. By using this approach, single molecule STED experiments can be carried out while the confocal control-experiment is performed simultaneously, allowing to account for measurement artifacts due to the high power of the STED laser. We will show examples from single molecule imaging, where blinking and bleaching are monitored using the confocal data. Furthermore, we will present STED-FCS data, where the confocal data allows insight into changes of the sample due to the STED laser. Since the control experiment for the influence of the STED laser is performed at the same time as the STED measurement, experimental parameters can be adjusted online to give highest resolution while ascertaining that the relevant information drawn from the experiment is not affected.

10071-30, Session PSun

Improved multi-parameter wide-field imaging and spectroscopy system

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Time-resolved fluorescence microscopy is a technology of significant interest for biological and biomedical applications. It is used to study cell morphology as well as to unravel cellular signalling pathways, protein folding, and interactions between biomolecules. Today the majority of systems for fluorescence lifetime imaging (FLIM) is based on confocal laser or sample scanning. Here, we present a different approach. Assembling a dedicated detector head with a segmented anode structure enables the construction of a time and spatial resolving single photon counting detection system. The photon to electron converting detector head contains a photocathode, a two stage microchannel plate stack, and a resistive layer for charge mirroring. The detection system is coupled onto a widefield fluorescence microscope. We implemented picosecond pulsed epi-fluorescence and total internal reflection excitation.

The detector records the time interval between an excitation pulse and a detected photon on a picosecond timescale, spatial information, as well as the macroscopic arrival time. A time resolution of less than 100 ps could be achieved. This fits well to most common organic dyes and all fluorescent proteins. It allows furthermore time-resolved FRET studies.

In this poster, we present recent improvements of the detection system to lower the dead time and to enhance the photon throughput. Since the system is free of scanning parts it is well suited for long term life cell imaging under low light level conditions.

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10071-31, Session PSun

PEGylated perylenemonoimide-dithienylethene for super-resolution imaging of liposomes

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We have designed and synthesized an amphiphilic photoswitchable dyad, PEGylated perylenemonoimide-dithienylethene (PEG-PMI-DTE), which exhibits evident photochromism and fluorescence switching of bistability. The structures of liposomes can be observed directly under super-resolution fluorescent microscopy by aid of the amphiphilic photoswitchable dyd as staining agent, with optical resolution about 50 nm.

10071-32, Session PSun

Enhanced simulator software for image validation and interpretation for multimodal localization super-resolution fluorescence microscopy

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Optical super-resolution techniques such as single molecule localization have become one of the most dynamically developed areas in optical microscopy. These techniques routinely provide images of fixed cells or tissues with sub-diffraction spatial resolution, and can be even applied for live cell imaging under appropriate circumstances. Localization techniques are based on the precise fitting of the point spread functions (PSF) to the measured images of stochastically excited identical fluorescent molecules. These techniques require controlling the rate between the on, off and the bleached states, keeping the number of active fluorescent molecules at an optimum value, so their diffraction limited images can be detected separately both spatially and temporally.

Because of the numerous (and sometimes unknown) parameters, the imaging system can only be handled stochastically. For example, the rotation of the dye molecules obscures the polarization dependent PSF shape, and only an averaged distribution – typically estimated by a Gaussian function – is observed. TestSTORM software was developed to generate image stacks for traditional localization microscopes, where localization meant the precise determination of the spatial position of the molecules. However, additional optical properties (polarization, spectra, etc.) of the emitted photons can be used for further monitoring the chemical and physical properties (viscosity, pH, etc.) of the local environment. The image stack generating program was upgraded by several new features, such as: multicolour, polarization dependent PSF, built-in 3D visualization. These features make the program an ideal tool for optimizing the imaging and sample preparation conditions.

10071-33, Session PSun

Real time In-situ super-resolution imaging of the self-assembly processes of block copolymers upon solvent vapour annealing (SVA)

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Super resolution imaging technique has timely made up the gap between the spatial drawback of optical microscope and temporal drawback of electron microscope. Recently, the emergence and development of fluorescent photoswitches, whose fluorescence could be controlled between

"on" and "off" by light, has enabled super resolution imaging of self-assemblies of block copolymers, organelle like lysosomes and electrospun nanowires. These results are solely focused on immobile objects due to the facts that the method is based on reconstruction from thousands of images. Very few investigations of real time in-situ imaging based on super-resolution imaging has been reported til now. On one hand, we can improve the imaging speed of a single reconstructed image by enhancing the fading speed while maintaining the fatigue resistance of photoswitch. On the other hand, we can also observe the dynamic procedures if they are slow enough. Solvent vapour annealing (SVA) technique has been widely used in preparation ordered nanostructures of block copolymers due to thermodynamic incompatibility of between their constituent blocks. However, real time in-situ visualization of block copolymer from one domain to another, which could not be realized by conventional optical microscope due to its resolution limit, has been generally relied on GISAXS analysis method.

Herein, we report the real time in-situ imaging the self-assembly of block copolymer (PS-b-PEO) upon solvent vapour annealing by using PMI-N-HABI as fluorescent probe. In a self-made chamber, the block copolymer PS-b-PEO mixed with PMI-N-HABI was spin-coated onto the bottom and the SVA procedure was triggered by placing a certain amount of solvent benzene in a container in the chamber. Continuous observation of the same position was realized and the reconstructed images were obtained based on controlling the fluorescence switching through 405 nm irradiation and intermittent recovery. Taking advantage of super resolution, we have obtained clearer images of how block copolymer transformed from disorder to highly order structures using optical microscope.

10071-34, Session PSun

Quantitative evaluation of the accuracy and variance of individual pixels in a scientific CMOS (sCMOS) camera for computational imaging

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"Scientific" CMOS (sCMOS) camera architecture fundamentally differs from CCD and EMCCD cameras. In scientific digital CCD and EMCCD cameras conversion from charge to the digital output is through a single electronic chain and the read noise and the conversion factor from photoelectrons to digital outputs are highly uniform for all pixels, although quantum efficiency may spatially vary.

In CMOS cameras, the charge to voltage conversion is separate for each pixel and each column has independent amplifiers and analog to digital converters, and pixel-to-pixel variation in quantum efficiency is possible. The "raw" output from the CMOS image sensor, includes pixel-to-pixel variability in the read noise, gain, offset and dark current. Scientific camera manufacturers digitally compensate the raw signal from the CMOS image sensors.

Unless corrected, inaccuracies and noise in image can introduce artifacts in computational imaging, such as light sheet microscopy or localization microscopy, especially for maximum likelihood estimation methods. We measured the distributions and spatial maps of individual pixel offset, dark current, read noise, linearity, photoresponse non-uniformity (PRNU) and variance distributions of individual camera pixels for a Hamamatsu ORCA Flash 4.0 sCMOS camera. Measurements are taken with highly uniform and controlled illumination over low light conditions from dark conditions and at multiple low light levels between ~20 to ~1,000 photons / pixel per frame.

We further show that using individual pixel variance to "correct" pixel gain for photo response non-uniformity (PRNU) correction is fundamentally incorrect and instead increases PRNU in cameras with accurate factory calibration.

10071-35, Session PSun

Dynamic SIM combined to optical sectioning for super resolution fluorescence imaging in living cells

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Live cell studies at the molecular level strongly depend on imaging performances. Structured illumination microscopy (SIM) brings a significant gain, as an increase of a factor 2 in lateral resolution allows observation of many new phenomena. However its current use for biological applications still requires major technical improvements in order to combine lateral super resolution with video rate fluorescence imaging and optical sectioning in living samples.

We present a structured illuminated microscope by fringe projection together with an original and efficient reconstruction algorithm that only requires 4 acquired images (instead of 9) to obtain a super resolution one. We also combine SIM with direct optical sectioning allowing imaging of in depth phenomena inside thick samples. Using those improvements and a sliding recombination of the raw images, it is possible to create super resolution movies with a quarter of the information renewed in each reconstructed image. This unique approach allows realizing dynamic SIM movies in live cells with high temporal resolution.

10071-36, Session PSun

3D astigmatic STORM imaging enhanced with robust method for background radiation estimation

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Althow several studies have shown that additional step of local background estimation in single-molecule superresolution data processing may increase the localization accuracy, such methods are still do not widespread, at least in literature. The most common state-of-the-art algorithm of background estimation is running median filter, which enables real-time computation and robustness to outliers, inherent to single-molecule superresolution data.

In this study we developed computationally efficient running mode filter, which enables robust estimation of background irradiation in any given pixel of the time-space raw data for single-molecule superresolution microscopy. Proposed running mode filter evaluates the most probable value of the intensity distribution in every pixel of the data as the most probable value (mode) of a heavy-tailed distribution. Under this concept local peakes in intensity time dependense in a pixel, caused by fluorophores switching affects only local distribution skewness, but not its mode. This local background estimates was utilized in superresolution data processing

in two ways. It was used for single fluorophore detection, decreasing both false-positive and false-negative detections. Obtained nonuniform background term was also introduced into maximum likelyhood estimator which increases the fidelity of parameters estimates, i.e. fluorophore position and fluorophores image shape parameters. We observed that fidelity enhancement was notably prominent for fluorophores image shape parameters estimates, which in case of astigmatic 3D STORM imaging leads to significant improvement of the resulting 3D superresolution image, especially in case of poor signal-to-background ratio.

Using Fourier Ring Correlation as the 3D localization accuracy metrics, we show that proposed background estimation technique is superior to running median filter, and both are superior to the most common locally uniform background assumption.

10071-37, Session PSun

Probing single processive molecular motors with high-speed optical tweezers and fluorescence microscopy

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Motor proteins are molecular machines that convert the chemical energy from ATP hydrolysis into mechanical work and movement. Among those, myosins are mechanoenzymes that interact with actin filaments to drive muscle contraction, control cell tension and transport material within the cell.

Optical techniques for the imaging and manipulation of single molecules provide high sensitivity, specificity, localization accuracy and time resolution, thus representing an optimal tool for studying molecular motors. In the last 20 years, a variety of single-molecule techniques has been developed to probe motor protein properties such as movement directionality, run length, step size, processivity mechanisms, rotational movements, and intramolecular structural changes.

Here we present a study of single processive myosin motors based on the combination of high-speed optical tweezers force spectroscopy and single molecule fluorescence imaging techniques. Ultra-fast force-clamp spectroscopy (Capitanio et al., Nature Methods 9, 1013-1019, 2012) is applied to study the dependence of the chemo-mechanical properties of myosin V motors on the applied load to reveal the mechanisms at the base of processive movement. On the other hand, single molecule localization through FIONA (Yilditz et al., Science 300, 2061-2065, 2003) is applied to in vitro motility assay to measure parameters such as the run length, velocity and step size of single myosin V motors, labelled with Quantum Dots, under unloaded conditions.

10071-38, Session PSun

Spatially encoded fast single molecule imaging with full-field of view

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Single-molecule fluorescence spectroscopy is one of the most powerful techniques for studying dynamics of complex bio-molecular systems. An array detector such as EMCCD camera has been a method of choice because of its capability to monitor multiple individual molecules simultaneously over long time, leading to thorough statistical analyses of events. However, the limited temporal resolution of the detector has hampered to study fast dynamics in a large field of view. Here, we present a novel method to improve the temporal resolution by 5 to 8 times in a simple way. By rotating one of the relay imaging mirrors during the exposure period, we encode high temporal information to several positions of the detector. We have demonstrated the feasibility of this method by monitoring the photo-blinking of organic dye, the binding and unbinding events of extremely short double stranded DNA and the conformational changes of Holliday junctions. Our results agree well with previous ones obtained from confocal microscopy which could only observe one molecule at a time. We expect our method could be applied to any camera-based system and it will play an important role in the field of fast single molecule imaging as well.

Conference 10072: Optical Diagnostics and Sensing XVII: Toward Point-of-Care Diagnostics

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10072-1, Session 1

Portable SERS sensor for malachite green and other small dye molecules

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Being able to carry out sensitive detection of specific chemicals on site can be extremely powerful in many fields. Owing to its molecular fingerprinting capability, surface-enhanced Raman scattering (SERS) has been one of the technological contenders. In this paper, we describe the novel use of DNA topological nanostructure on nanoporous gold nanoparticle (NPG-NP) array chip for smart chemical sensing. DNA molecules have been heavily exploited as bio-recognition elements for their in technologies such as microarray, polymerase chain reaction, genomic sequencing and etc. The formation and evolution of double-stranded DNA beta-helical nanostructures can be effectively reported by either pre-labeled oligonucleotides, or post-labeling using intercalating fluorescence dyes. Similarly, surface-enhanced Raman spectroscopy (SERS) has also been employed to report the Watson-Crick hybridization and melting of two complementary oligonucleotides.

NPG-NP array chip showcases tunable pore and ligament sizes ranging from nanometers to microns. The nanoporous structure and sub-wavelength nanoparticle shape contribute to its unique LSPR properties. NPG-NP features large specific surface area and high-density plasmonic field enhancement known as “hot-spots”. Hence, NPG-NP array chip has found many applications in nanoplasmonic sensor development. In our recent studies, we have shown that NPG-NP array chip can be utilized for high-sensitivity detection by various enhanced spectroscopic modalities, as photothermal agents, and for disease biomarker detection.

In this paper, we explore the potential of using non-double helical DNA nanostructures for malachite green and small molecule sensing. This technique can provide novel label-free molecular sensing capability with high sensitivity and specificity.

10072-2, Session 1

Optimization of SERS assay for the transition from benchtop to handheld Raman systems

Monika Schechinger, Haley L. Marks, Texas A&M Univ. (United States); Mahua Choudhury, Irma Lerma Rangel College of Pharmacy, Texas A&M Univ. (United States); Gerard L. Coté, Ctr. for Remote Healthcare Technology, Texas A&M Univ. (United States)

Human biomarkers are indicative of the body’s relative state prior to the onset of disease. The ability of physicians to detect biomarkers such as microRNA-17, a potential indicator of preeclampsia in pregnant woman, can enable early diagnosis and preventive intervention. While blood biomarker detection has achieved considerable success in laboratory settings, its clinical application is lagging and commercial point-of-care devices are rare. One approach explored uses surface enhanced Raman spectroscopy (SERS)with nanoparticles functionalized to detect microRNA-17 and requires large and expensive benchtop Raman microscopes. However, strides have been made in developing portable Raman systems for field applications. In comparing benchtop and portable Raman, characteristics of the SERS assay were explored to strengthen the plasmonic effect responsible for enhancing the Raman spectrum. The Raman spectrum and intensity of three different photoactive molecules were compared as potential Raman reporter molecules. Furthermore, the characteristics governing the formation of SERS colloidal nanoparticle assemblies in response to DNA/miRNA binding were investigated. Factors such as shifts in the local surface plasmon resonance (LSPR) wavelength and photoactive dyes impacted the SERS enhancement capabilities for portable Raman systems. Additionally, the use of short stabilizing hexa (ethylene) glycol spacer between the nanoparticle and capture ligand reduces the inter-particle distance and enhances the SERS response to microRNA-17 using both 532 nm and 780 nm excitation lasers. Therefore, the use of chromophores and short chain spacers to increase aggregation and SPR for optimizing Raman spectra may be a key in transitioning SERS biomarker detection from laboratory testing to point-of-care biomarker detection.

10072-3, Session 1

SERS-based point-of-care assay to detect cardiac troponin I

Javier T. Garza, Gerard L. Cote, Po-Jung Huang, Jun Kameoka, Texas A&M Univ. (United States); Samuel Mabbott, Duncan Graham, Univ. of Strathclyde (United Kingdom)

The accurate and timely detection of cardiac biomarkers is an important tool to diagnose an acute myocardial infarction and provide the necessary information to apply the appropriate treatment. Ideally, the measurement would be performed as soon as the patient indicates symptoms of a heart attack. Therefore, there is a need for a portable point-of-care device that can measure cardiac biomarkers with the appropriate sensitivity and precision. Surface enhanced Raman spectroscopy (SERS) is a sensitive optical technique that can be used in biochemical assays to quantify analytes. In this work, a SERS based point-of-care device was developed to measure cardiac troponin I. Nanostars were synthesized and functionalized with a Raman reporter molecule and an anti-cardiac troponin I antibody. Magnetic nanoparticles were also synthesized and functionalized with a second anti-cardiac troponin I antibody. The particles were stabilized with silica and polyethylene glycol (PEG). The assay was tested on a magnetic microfluidic chip to determine different concentrations of troponin. In the assay, the SERS spectrum intensity was proportional to the amount of troponin present.

10072-4, Session 1

SERS of deceased donor urine as a means of predicting the performance of kidney transplant

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Successful transplantation of kidneys from deceased donors requires the use of kidneys whose quality is adequate. This presentation reports the potential utility of SERS to assess the quality in conjunction with available clinical information. SERS was conducted on the urine samples of deceased kidney donors classified to three groups that exhibit the following distinct features: donors with acute tubular necrosis (ATN), recipients showing delayed graft function (DGF) and control (no ATN nor DGF) of deceased kidney donors (n=10 for each group, total n=30). 10 SERS spectra were acquired for each urine using 632.8 nm laser and a total of 300 spectra were analyzed using principal component analysis (PCA) followed by linear discriminant analysis

(LDA) and quadratic discriminant analysis (QDA) for spectra classification. 10-fold cross validation was applied on both classifier to evaluate their performance. Promising discrimination was observed for all groups. The sensitivity and specificity of PCA-LDA classifier for control, ATN, and DGF groups are 81.00%, 92.10%; 91.00%, 82.00% and 87.00, 85.29% respectively. The ability of SERS analysis of deceased donor urine samples to provide early indication of transplant outcomes will prove extremely valuable for surgeons in choosing graft with higher potential survival rate and thus reduce graft failure rate and transplant cost.

10072-5, Session 1

Nanoimprint lithography-based plasmonic crystal-surface enhanced Raman scattering substrate for point of care testing application

Tatsuro Endo, Osaka Prefecture Univ. (Japan); Kenji Yamada, Osaka Univ. (Japan)

Surface enhanced raman scattering (SERS) is known for its high sensitivity toward detection down to single molecule level under optimal conditions using surface plasmon resonance (SPR). To excite the SPR for SERS application, nanostructured noble metal supports such as a nanoparticle have been widely used. However, for excitation of SPR for SERS application using noble metal nanoparticle has several disadvantages such as sophisticated fabrication procedure and low reproducibility of SPR excitation efficiency. To overcome these disadvantages, in this study, plasmonic crystal (PC)-SERS substrate which has a periodic noble metal nanostructure was successfully fabricated rapidly and cost-effectively based on nanoimprint lithography (NIL).

For fabrication of PC-SERS substrate based on NIL, gold layer (20-200 nm) was deposited onto triangular configurated hole array polymer mold (hole diameter and distance: 230 nm, and depth: 200 nm) using thermal evaporator. And then, the gold layer was mechanically peeled off using thermosetting resin. Based on these fabrication procedure, PC-SERS substrate was obtained with high reproducibility.

To evaluate the SERS performance using PC-SERS substrate, detection of phenobarbital (PB) which have been used as an antiepileptic drug was carried out using commercially available handy type raman module (laser wavelength: 785 nm, power: 50 mW or 3 mW). As a result, specific raman spectrum of PB could be observed at down to 0.01 mmol/L. This result shows that the PC-SERS substrate and handy type raman module has great potential for high sensitive detection of target molecules in point of care testing application.

10072-6, Session 2

Innovative chip-based sample preparation strategies suitable for the Raman microspectroscopic identification of bacteria

Susanne Pahlow, Friedrich-Schiller-Univ. Jena (Germany) and Leibniz-Institut für Photonische Technologien e.V. (Germany); Stephan Stöckel, Friedrich-Schiller-Univ. Jena (Germany); Uwe Hübner, Leibniz-Institut für Photonische Technologien e.V. (Germany); Petra Rösch, Friedrich-Schiller-Univ. Jena (Germany); Dana Cialla-May, Karina Weber, Jürgen Popp, Friedrich-Schiller-Univ. Jena (Germany) and Leibniz-Institut für Photonische Technologien e.V. (Germany)

Raman microspectroscopy is an attractive tool for identifying microorganisms on single cell level in a fast and reliable manner based on the specific chemical information provided by the Raman spectra. Compared to cultivation based approaches, which are still commonly used for the identification of bacterial cells, the time span necessary for obtaining the test result can be significantly reduced. However, many applications require a suitable sample preparation prior to the Raman spectroscopic investigation since most sample matrices, i. e. body fluids or soil, contain Raman active components, which will interfere with the bacterial spectra and hamper a correct identification.

We have developed a chip based system, which allows isolating bacterial cells and investigating them subsequently via Raman spectroscopy on the same platform. The Raman chip can be modified with capture probes depending on the desired application. Since the identification is achieved via the specific bacterial Raman spectra, capture molecules which target a broad range of species such as antibodies, siderophores or antibiotics are the preferential choice. Due to the wide spectrum of bacterial species which can be accessed, the applicability of the system in the biomedical field is accordingly diversified and includes, for example, the detection of sepsis relevant pathogens or monitoring drinking water for microbial contamination.

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10072-7, Session 2

Particle-based isolation and detection of microorganisms in biophotonics

Karina Weber, Leibniz-Institut für Photonische Technologien e.V. (Germany) and Friedrich-Schiller-Univ. Jena (Germany); Susanne Pahlow, Sandra Kloss, Friedrich-Schiller-Univ. Jena (Germany); Dana Cialla-May, Leibniz-Institut für Photonische Technologien e.V. (Germany); Jürgen Popp, Leibniz-Institut für Photonische Technologien e.V. (Germany) and Friedrich-Schiller-Univ. Jena (Germany)

The identification of microorganisms is of great interest for manifold applications e.g. the detection of infectious diseases or for the evaluation of soil remediation and recultivation methods. As powerful tool, optical detection schemes like vibrational spectroscopic techniques are discussed within the context of biophotonics. However, when investigating microorganisms in complex matrices, via Raman spectroscopy for example,the overall spectrum might be dominated by contributions from matrix components, which hinder the reliable detection and identification of bacteria. Therefore, a fast and simple isolation strategy is important. Within this contribution, particle-based isolation strategies suitable for a subsequent Raman-based detection will be introduced. These techniques facilitate separating the microorganisms from the complex matrix, accordingly the occurrence of background signals is dramatically reduced. For example, polyethyleneimine modified polyethylene particles as well as amine modified expanded glass particles enable pH controlled/directed binding and release of bacteria. The advantages of this approach include simple handling, a high enrichment efficiency, and the preservation of viable bacteria. Finally, the isolation of bacteria from medical (e.g. urine, sputum) and environmental samples (e.g. soil) is demonstrated followed by a reliable Raman-based identification of various species on single cell level.

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10072-8, Session 2

Mid-IR spectroscopic instrumentation for point-of-care diagnosis using a hollow silica waveguide gas cell

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Laser spectroscopy provides the basis of instrumentation developed for the quantification of biomarkers associated with infectious disease. Bacterial presence and activity can be identified by the mixture of volatile organic compounds (VOCs) that they produce. Variations in microbial populations can be associated with a range of common and serious medical conditions. This includes gastrointestinal diseases such as Clostridium difficile infection and inflammatory bowel disease, plus lung infections such as Pseudomonas aeruginosa. The aim of our instrumentation is to determine VOC concentrations through spectroscopic analysis of the headspace of biological fluids, or exhaled breath. This can allow diagnosis at point-of-care, at much faster timescales than current tests, and without the need to transport samples to an external laboratory.

The technology is centred on a multi-channel pulsed quantum cascade laser system that allows multiple lasers with different wavelengths to be used simultaneously, each selected to monitor a different diagnostic biomarker. The instrument also utilizes a hollow silica waveguide (HSW) gas cell which has a very high interaction pathlength to internal volume ratio. This allows sensitive detection of low volume gas species from small biological samples. The spectroscopic performance and response of a range of HSW gas cells with different lengths and bore diameters has been assessed using methane as a test gas. The results have been compared with those obtained using a multi-pass Herriot cell. A prototype instrument has been built and approved for clinical trials for detection of infection in acute-care patients via analysis of ventilator breath.

10072-9, Session 2

Diagnosis of bacterial infections from a small volume of blood using a waveguide-based optical biosensor

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In sub-Saharan Africa, bacterial infections are a leading cause of morbidity and mortality. Many factors complicate diagnosis, including the presence of multiple infections in a single patient and lack of infrastructure in rural settings. In pediatric patients, an additional challenge lies in low sample volume due to anemia and dehydration. To address these issues, we have developed novel diagnostic assays on a waveguide-based biosensor platform, to rapidly and specifically identify pathogen biomarkers from a small sample of patient blood. For the detection of Salmonella, we use a method called lipoprotein capture, which exploits the nature of lipoproteins to sequester pathogen biomarkers from blood. In this presentation, we will describe our waveguide platform, the novel assay architecture and protocol for lipoprotein capture for Salmonella detection, and our modifications to address small volume samples. Our studies of pediatric samples will also be discussed, and we will present novel ideas and current work on other biomarkers.

10072-10, Session 3

The light at the service of medicine: optical sensing beside the patient's bed

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The last twenty years have seen the increasing demand by physicians of devices able to carry out fast and reliable measurements of chemical and biochemical parameters beside the patient's bed so as to allow the formulation of a rapid and reliable diagnosis and/or the choice of the most appropriate therapy, avoiding the need for analysis of centralized laboratories. These are the so-called Point of Care Testing (POCT) devices that are becoming essential for the analysis of many diseases, where a quick medical attention is crucial for the patient's life.

Optical biosensors and chemosensors can definitely play a fundamental role in this area and the use of optical fibers as optical links can also lead to invasive continuous measurements within the human body. The determination of one single parameter is sometimes sufficient, but it is important to emphasize that it is often necessary to monitor a panel of biomarkers associated to the onset and/or to the development of a definite pathology and, in this context, the optical biochip can play an essential role in the development of POCT equipment. The activity developed at the Institute of Applied Physics in this field in strict collaboration with physicians is described with particular attention to the measurement of bile-containing refluxes in the gastroesophageal apparatus in non- hospitalized patients, to the detection of gastric carbon dioxide in intensive care patients, to the simultaneous measurement of sepsis biomarkers in serum samples and to the measurements of immuno-suppressants in transplanted patients.

10072-11, Session 3

Novel fluorescence-based POCT platform for therapeutic drug monitoring in transplanted patients

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A novel therapeutic drug monitoring point of care testing (POCT) optical device for the detection of immunosuppressants in transplanted patients

was designed and tested, with the body interface constituted by an intravascular microdialysis catheter (MicroEye®) which provides the dialysate as clinical sample. An optical biochip with 10 microchannels, based on total internal reflection fluorescence (TIRF), enables the frequent measurement of immunosuppressants. Heterogeneous competitive immunoassays for the detection of mycophenolic acid, tacrolimus and cyclosporine A are implemented on the different microchannels, with the derivative of the immunosuppressants immobilised on the bottom part of the micro-channels.

10072-12, Session 3

Reliable glucose monitoring by ex-vivo blood microdialysis and infrared spectrometry for patients in critical care

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Blood glucose monitoring has been realised by biosensors in combination with micro-dialysis, using either intravascularly or subcutaneously implanted catheters. Another alternative is ex-vivo micro-dialysis of continuously sampled heparinised whole blood available from ICU patients. However, most devices suffer from inaccuracies due to variable recovery rates. Infrared spectrometry has been suggested for analyte detection and quantification, since besides glucose other clinically relevant analytes can be simultaneously determined that are, e.g., important for intensive care patients. Perfusates with acetate and mannitol have been investigated as recovery markers (internal standards). Despite the overlap of mannitol and glucose spectra, their simultaneous accurate quantification by infrared spectrometry was successful, which included also other low-mass components such as lactate and urea, contributing to the dialysate spectra. In contrast to the previously used acetate as marker substance, an almost linear dependency between mannitol loss and glucose recovery was observed for micro-dialysis catheters using human serum as exemplary body fluid; similar results were obtained when testing two flat membranes within a custom-made micro-dialysator using porcine heparinized whole blood. By this, a straightforward compensation of any dialysis recovery rate variation during patient monitoring is possible. The combination of microdialysis with infrared spectrometry provides a calibration-free assay for accurate continuous glucose monitoring, as reference spectra of dialysate components can be a-priori allocated. Using this system, glucose concentration values in whole blood can be reliably and continuously monitored, as such measurements can be considered as the gold standard in glycemic control of critically ill patients.

10072-13, Session 3

Monitoring of interstitial buffer systems using micro-dialysis and infrared spectrometry

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Nowadays, sensing systems, especially for blood glucose monitoring, are important point-of-care devices for the hospital and personalized diabetes technology. FTIR-spectrometers have been successfully employed for the development of continuous bed-side monitoring systems designed in combination with micro-dialysis. In-vivo applications for critically ill patients can be envisaged, especially as further analytes and parameters are accessible, which are of interest for intensive care such as lactate, urea, pCO2 and pH. The human body maintains the blood pH around 7.4, but for severe pH level changes acidosis or alkalosis can lead to serious health problems. Three different buffer systems exist based on bicarbonate, phosphate and proteins. Transmission infrared spectra of pH-dependent buffer components were recorded for the most important bicarbonate system. Besides a spectrometer, the developed technology includes a micro-dialysis probe and a fluidic system. By using the CO2 and HCO3- bands of the bicarbonate spectra, the pH of the harvested biofluid can be predicted using the Henderson-Hasselbalch equation. Furthermore, we studied the solubility of CO2 in aqueous solutions using gas mixtures of N2 and CO2 with known composition within partial pressures of CO2 as relevant for in-vivo conditions. Thus, values of pCO2 up to 150 mm Hg (200 hPa) with distilled water and a Ringer solution, which is an isotonic electrolyte solution used for medical infusion, were measured at 25 °C and 37 °C (normal body temperature).

10072-14, Session 3

A LSPR fiber optic biosensor for point-of-care diagnostics

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For point-of-care (POC) of patients, sensitive, high-throughput biosensing platforms for identification and quantification molecular markers hold promise. Moreover, robust, low-cost, portable, highly-sensitive, and label-free devices that can analyze biofluids in a clinical setting, allowing on-the-spot detection, diagnosis, therapy efficacy, and prognosis are needed.

In regard to label-free techniques, localized surface plasmon resonance (LSPR) based nanobiosensors are considered one of the most powerful tools in the biosensor field. Incorporation of LSPR to fiber optics (LSPR-FO) offers advantages by avoiding the use of bulky optics and high-precision mechanics and could enable development of a compact handheld biosensing device for rapid, real-time detection of protein biomarkers, antigens, or analytes. Thus, we have developed an LSPR-FO nanoprobe for POC biosensing applications. For designing this LSPR nano-disk array, finite-difference, time-domain numerical methods were used, and electron-beam lithography and a metal lift-off process were applied for fabrication of the fiber end facets. A gold nano-disk square array with a diameter of 160 nm, a thickness of 35 nm, and a periodicity of 400 nm generated well-defined LSPR responses in response to white incident light. The gold nano-disk array was fabricated onto the fiber tips (4 or 9 μm diameter). The measured refractive-index (RI) sensitivity of the LSPR-FO nanoprobe was ~226 nm/RI units. LSPR-FO nanoprobes based on antibody-antigen interactions were used for measurement of a cancer biomarker, free-prostate specific antigen (fPSA), with a limit of detection of ~100 fg/mL (~3 fM). Further validation of this platform for detection of several other cancer biomarkers is in progress.

10072-15, Session 4

Toward unstained cytology and complete blood counts at the point of care

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Cytology tests, whether performed on body fluids, aspirates, or scrapings are commonly used to detect, diagnose, and monitor a wide variety of health conditions. Complete blood counts (CBCs) quantify the number of red and white blood cells in a blood volume, as well as the different

types of white blood cells. There is a critical unmet need for an instrument that can perform CBCs at the point of care (POC), and there is currently no product in the US that can perform this test at the bedside. We have developed a system that is capable of tomographic images with sub-cellular resolution with consumer-grade broadband (LED) sources and CMOS detectors suitable for POC implementation of CBC tests. The systems consists of cascaded static Michelson and Sagnac interferometers that map phase (encoding depth) and a transverse spatial dimension onto a two-dimensional output plane. Our approach requires a 5 microliter sample, can be performed in 5 minutes or less, and does not require staining or other processing as it relies on intrinsic contrast. We will show results directly imaging and differentiating unstained blood cells using supercontinuum fiber lasers and LEDs as sources and CMOS cameras as sensors. We will also lay out the follow up steps needed, including image segmentation, analysis and classification, to verify performance and advance toward CBCs that can be performed bedside and do not require CLIA-certified laboratories.

10072-16, Session 4

Field performance of a low-cost and fully-automated blood counting system operated by trained and untrained users

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Current flow-based blood counting devices require expensive and centralized medical infrastructure and are not appropriate for field use. In this paper we report a method to count red blood cells, white blood cells as well as platelets through a low-cost and fully-automated blood counting system. The approach consists of using a compact, custom-built microscope with large field-of-view to record bright-field and fluorescence images of samples that are diluted with a single, stable reagent mixture and counted using automatic algorithms. Sample collection is performed manually using a spring loaded lancet, and volume-metering capillary tubes. The capillaries are then dropped into a tube of pre-measured reagents and gently shaken for 10-30 seconds. The sample is loaded into a measurement chamber and placed on a custom 3D printed platform. Sample translation and focusing is fully automated, and a user has only to press a button for the measurement and analysis to commence. Cost of the system is minimized through the use of custom-designed motorized components. We performed a series of comparative experiments by trained and untrained users on blood from adults and children. We compare the performance of our system, as operated by trained and untrained users, to the clinical gold standard using a Bland-Altman analysis, demonstrating good agreement of our system to the clinical standard. The system's low cost, complete automation, and good field performance indicate that it can be successfully translated for use in low-resource settings where central hematology laboratories are not accessible.

10072-17, Session 4

Development of an imaging method for quantifying a large digital PCR droplet array

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Portable devices have been recognized as the future linkage between end users and a lab-on-a-chip device. It has a user friendly interface and can provide apps to interface headphones, cameras, and communication duct. In particular, the digital resolution of cameras installed in smartphones or pads already has a high imaging resolution with a high number of pixels. It has triggered researches to integrate optical fixtures with smartphone to provide microscopic imaging capabilities. Furthermore, smartphones are also applied to perform colorimetric strip tests. In this paper, we report our study on developing a portable diagnostic tool based on the imaging system of a smartphone and a digital PCR biochip. A computational algorithm is developed to processing optical images taken from a digital PCR biochip with a smartphone in a black box. Each reaction droplet is recorded in pixels and can be analyzed in a sRGB (red, green, and blue) color space. Each pixel is first calibrated by using a reference color temperature by a color transfer model. The color transfer matrix is retrieved by using a reference color chart and calculated by Least-Square method. Multistep filtering algorithm and auto-threshold algorithm are adopted to minimize background noise contributed from ccd cameras and rule out false positive droplets, respectively. Finally, an size-filtering method is applied to identify number of positive droplets to quantify target's concentration. Statistical analysis is then performed for diagnostic purpose. This process can be integrated in an app and can provide a user friendly interface without professional training.

10072-18, Session 4

DotLens smartphone microscopy for biological and biomedical applications

Yu-Lung Sung, Fusheng Zhao, Wei-Chuan Shih, Univ. of Houston (United States)

Recent advances in inkjet-printed optics have created a new class of lens fabrication technique. Lenses with a tunable geometry, magnification, and focal length can be fabricated by dispensing controlled amounts of liquid polymer onto a heated surface. This fabrication technique is highly cost-effective, and can achieve optically smooth surface finish. Dubbed DotLens, a single of which weighs less than 50 mg and occupies a volume less than 50 ?L. DotLens can be attached onto any smartphone camera akin to a contact lens, and enable smartphones to obtain image resolution as fine as 1 μm. The surface curvature modifies the optical path of light to the image sensor, and enables the camera to focus as close as 2 mm. This enables microscopic imaging on a smartphone without any additional attachments, and has shown great potential in mobile point-of-care diagnostic systems, particularly for histology of tissue sections and cytology of blood cells. DotLens Smartphone Microscopy represents an innovative approach fundamentally different from other smartphone microscopes.

In this paper, we describe the application and performance of DotLens smartphone microscopy in biological and biomedical research. In particular, we show recent results from images collected from pathology tissue slides with cancer features. In addition, we show performance in cytological analysis of blood smear. This tool has empowered Citizen Science investigators to collect microscopic images from various interesting objects.

10072-19, Session 4

Hybrid integration of VCSELs onto a silicon photonic platform for biosensing application

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Recently major research efforts have started to focus on using silicon photonics as a potential platform for biosensing application, as it has benefited from re-deploying established complementary-metal-oxide-semiconductor (CMOS) foundry for manufacturing on high volume. On the other hand, life sciences industry needs emerging technologies to revolute existing instrumentation. For example, the implantable sensor for continuous glucose monitoring is of great interest. It provides an alternative of a better and safer glycaemic control for the patients to the disposable strip. An optical approach based on integrated silicon photonics device is an attractive option because it exploits the unique asset of extreme

miniaturization of silicon photonics. A major challenge to realize fully functional silicon photonics is integrating the miniaturized light source on-chip.

In this paper, we presented an approach of directly integration a laser onto a silicon photonics device. A vertical-cavity-surface-emitting-laser (VCSEL) was flip-chip bonded onto a grating coupler in a silicon photonic device. By engineering the reflow of the solder balls used for electrical and mechanical bonding, the VCSELs were bonded at 10? to achieve the optimum angle-of-incidence to the planar grating coupler. A coupling efficiency measured was -5.6 dB at 1547 nm from the passive flip-chip VCSEL-to-grating, while an active alignment gave an efficiency of -4.6 dB for a perfectly aligned structure. The 1 dB discrepancy between optical loss values confirmed that the general purpose of the flip-chip design concept is achieved. This flip-chip approach to integrate a light source on chip opens the possibly of highly compact sensor system.

10072-20, Session 4

Wearable see-through ophthalmoscope for point of care

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There is an increasing demand for point of care diagnostics which is driving the development of portable ophthalmoscopes. Here we study the proof of concept of a wearable see-through ophthalmoscope. A wearable head-mounted system offers the advantage of an easier alignment and a more stable acquisition process, due to its independence on head movements. We use holographic components to provide a see-through design: a system able to avoid the obstruction of the user's field of view typical of classic ophthalmoscopes.

The performances of the device are investigated in detail, such as: optical resolution, contrast and energy budget. The study is performed with ray-tracing simulations and their validity is tested with experimental results on ex-vivo human eyes.

Final discussion focuses on the possibility of extending this technology to continuous monitoring. Indeed, the characteristic of see-through can be used for chronic measurements without disturbing the user's activity. Possible targets for those measurements are retinal blood vessels for extracting information about body health, and gaze direction.

10072-39, Session PMon

Pulsed photoacoustic non-invasive glucose measurement based on dual-wavelength near infrared differential-absorption method

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Non-invasive, portable, economical, dynamic blood glucose monitoring device has become a functional requirement for diabetes in his regulating entire life. In photoacoustic (PA) dual-wavelength near infrared differential-absorption measurement, the glucose in aqueous solutions was excited by two short duration (order of nanoseconds) diode laser pulse system which wavelengths were 808nm and 950nm, respectively. The glucose in aqueous solutions to be analyzed was contained within the first photoacoustic cell (PAC), whereas the presence of a second reference photoacoustic cell (PAC) provided energy monitoring.

The PA pulsed signals were measured using a piezoelectric detector and the voltage pulses from the piezoelectric detector were amplified with a precision AC 9452 amplifier, the PA signal strength (PSS) was computed as the integration of the Hilbert Transform of the PA signal. To investigate the possible contributions of other blood analytes to the total photoacoustic response from blood, solutions of sodium chloride, cholesterol, and Bovine Serum Albumin (BSA) were investigated. The function generation and signal acquisition were performed in the PC using Labview software.

The experimental results showed that the photoacoustic (PA) dual-wavelength near infrared differential-absorption measurement can be engineered to detect blood glucose. The results of our study have the potential to not only better develop photoacoustic (PA) blood glucose meter, but also extend the viability and use of photoacoustics into detection of otherwise blood components.

10072-40, Session PMon

An improved optical scheme for self-mixing low-coherence flowmeters

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Several well established optical techniques are nowadays available for flow measurement. As an example, laser Doppler velocimetry (LDV) is widely used both in industrial and laboratory applications.

However, in many applications the fluid is turbid and/or the duct is buried in a diffusive medium thus giving rise to multiple-scattering regime hence limiting the applicability of measurement techniques based on coherent sources.

Low-coherence techniques such as Doppler optical coherence tomography (DOCT) can be used to overcome such limitation.

Nevertheless, despite the advantages offered by LDV and DOCT techniques, some drawbacks such as complexity and costs, limit their field of applications.

Mainly for coherent techniques, system complexity and cost can be significantly reduced by exploiting the self-mixing (SM) approach in which a portion of the light emitted by the source is backscattered from the moving scatterers and then directly detected by the back-faced monitor photodiode generally included in the source package.

In our recent paper we described a low-coherence system based on a common-path optical scheme able to fully exploit the advantages offered by the SM approach, thus capable to avoid the need of the reference arm usually required by low coherence techniques to set the measuring region.

In this paper, we present an improved optical scheme based on optical fibers.

The developed measuring system has been demonstrated to be able to directly measure the flow in pipes.

10072-41, Session PMon

Optical sensor embedded automatic cupping system for personalized cupping therapy

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There are many automatic cupping systems available in the market, but they are not designed to optimize the efficacy of cupping therapy. In this study, we developed a homemade automatic cupping system with optical sensor embedded cup for personalized cupping therapy. For the system, DC air pump which can provide maximum 200kpa vacuum and solenoid valves are connected to a cup through silicon tube to apply and to release negative

pressure inside of cup. Optical sensor consists of a dual wavelength LED which emits near infrared light (735nm and 850nm) and a photodiode. This sensor is placed at the center of cup inside by using a spring to keep a good contact with skin during cupping therapy. To observe the effect of cupping therapy, oxyhemoglobin and deoxyhemoglobin concentration changes were monitored by this sensor. Several cupping therapy experiments were conducted at the site of belly and waist to develop a feedback algorithm for personalized cupping therapy. Oxyhemoglobin concentration was immediately increased and maintained while deoxyhemoglobin concentration was slightly decreased when negative pressure was applied. Both oxyhemoglobin and deoxyhemoglobin concentrations returned to the baseline level when the solenoid valve released the negative pressure. The feedback algorithm is programed on a microcontroller to control a DC air pump and a solenoid valve based on the level of oxyhemoglobin. Clinical investigation is required to prove the usefulness of this automatic cupping therapy system.

10072-42, Session PMon

Design verification of a compact system for detecting tissue perfusion using bimodal diffuse optical technologies

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It is essential to monitor tissue perfusion during and after reconstructive surgery, as restricted blood flow can rapidly result in graft failures. Current clinical procedures to monitor tissue perfusion are insufficient, as they are intermittent and often subjective. To address this unmet clinical need, a compact, low-cost, multimodal diffuse correlation spectroscopy and diffuse reflectance spectroscopy (DCS-DRS) system was developed. The non-invasive system utilizes fiber-optic probes embedded in adherent skin patches to detect tissue perfusion in an automated and continuous fashion.

In this study, we verified DCS-DRS system performance by designing tissue simulating phantoms and developing experimental protocols for rigorous bench testing. Quantitative data analysis methods were employed and tested to enable the extraction of several perfusion parameters, including blood flow parameters and hemoglobin concentration and saturation.

For the DCS sub-system, a variety of flow phantoms were constructed assess the accuracy of the sub-system to estimate flow parameter. For the DRS sub-system, a set of liquid phantoms with varying scattering and absorption coefficients over a range of physiological relevant values were manufactured to test accuracy using an inverse model. We anticipate this design verification study will assure data integrity and prompt future pre-clinical and clinical studies.

10072-21, Session 5

340nm UV LED excitation in time-resolved fluorescence system for europium-based immunoassays detection

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In immunoassay analyzers for in-vitro diagnostics, a Xenon flash lamp has been widely used as excitation light source. Recent advancements in LEDs and its advantages over the flash lamps such as smaller footprint, better wall-plug efficiency, narrow emission spectrum, and no significant afterglow, have made them attractive light sources for gated detection systems. In this paper, we report on the design and implementation of a 340 nm UV LED based time-resolved fluorescence system based on europium as fluorescent marker. The optical excitation system is designed to collect up to 80% of the LED light emitted by the Lambertian emitter (1x1 mm2), and forms an image in the test cup with a magnification of 5. The fluorescence light emitted at 616 nm is collected by the detection system and focused onto a photomultiplier's photocathode operating in a photon-counting mode. The system performance was tested with the immunoassay based on the cardiac marker, TnI (200 ng/L). The same signal-to-noise ratio as for the flash lamp based system was obtained, operating the LED below specified maximum current. The background counts of the system and its main contributors were measured and analyzed. The signal-to-background ratio of the LED based unit was improved by a factor of two compared to that of the Xenon flash lamp based unit, due to the LEDs narrower emission spectrum and absence of afterglow. Key parameters of the LED system are discussed to further optimize the signal-to-noise ratio, and hence the sensitivity of the instrument.

10072-22, Session 5

Fluorescence analysis of ubiquinone and its application in quality control of medical supplies

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The presence of antioxidant issues such as redox potential imbalance in human body is a very important question for modern clinical diagnostics. The application of fluorescence analysis for quality control of medical supplies have got wide distribution in pharmacy. Implementation of fluorescence analysis into optical diagnostics of such wide distributed in a human body antioxidant as ubiquinone is one the steps for development of the device with a view to clinical diagnostics of redox potential. Recording of fluorescence was carried out with spectrometer using ultraviolet irradiation source with thin band (max at 280 nm) as a background radiation. Recording data was processed using statistical analysis and calculation of sensitivity which this equipment can provide for fluorescence analysis. Concentrations of ubiquinone from 0.25 to 2.5 mmol/l in explored samples were used for investigation of redox potential in wide range that covers oxidative stress and reductive stress phenomena. Principal analysis of fluorescence behavior for ubiquinone solution depends on concentration of antioxidant in oil and alcohol as a part of processing technics is presented. The sensitivity of this analysis allow to investigate fluorescence of ubiquinone solutions as well as similar antioxidants in human blood in vitro. As a technic in clinical diagnostics fluorescence analysis with processing method including fluorescence order analysis, it is step forward towards redox potential calculation and quality control in pharmacy for better health care.

10072-23, Session 5

Effect of sample bias on accuracy of a three-part leukocyte differential test when using AO-induced fluorescence and colorimetric features

Amy J. Powless, Timothy J. Muldoon, Univ. of Arkansas (United States)

A three-part leukocyte differential is a blood test that counts three distinct cell populations (granulocytes, lymphocytes, and monocytes), and

perturbations in these counts could indicate myriad clinical abnormalities, including sepsis, malignancy, or infection. Many point-of-care (POC) blood analyzers performing a leukocyte differential use acridine orange (AO) as a fluorescent contrast agent due to its colorimetric features unique to each population based on the red shift in AO emission that occurs with increased acidic environments within a cell. However, time-dependent red shift can limit image acquisition and thus sample size which could cause test inaccuracy. We have developed an automated differential algorithm based on the red-to-green fluorescence intensity ratio of the cells with fixed thresholds for each population. We used Bland-Altman analysis to quantify the accuracy. Our previous work has demonstrated that a red shift occurs in the span of two minutes, leading to inaccurate differential results. The reduced acquisition time could introduce sampling bias from the lack of available cells to count, especially in monocytes representing 4-10%. We have investigated the effect of sample bias on test accuracy when using AO-induced fluorescence and colorimetric analysis. The POC device we have developed can count an average of 200 cells per field-of-view (FOV) measuring 683x512μm and has produced results within +/-15% of a clinical system using six manually acquired FOVs acquired within a one minute window following a 5 minute incubation period. Our results indicate increased test variability in repeat measures per subject for one FOV compared to six FOVs.

10072-24, Session 6

Using consumer-grade devices for multi-imager non-contact imaging photoplethysmography

Justin R. Estepp, Air Force Research Lab. (United States); Ethan B. Blackford, Ball Aerospace & Technologies Corp. (United States)

Imaging photoplethysmography is a technique through which the morphology of the blood volume pulse can be obtained through non-contact video recordings of exposed skin with superficial vasculature. The acceptance of such a convenient modality for use in everyday applications may well depend upon the availability of consumer-grade imagers that facilitate ease-of-adoption. In this work, we consider the use of multiple, synchronous imagers and the effects of video compression as two features of imaging devices that may need to be examined when choosing an imager for this use. Multiple imagers have been used in several concept demonstrations and can lead to improvements in quality of the extracted blood volume pulse. However, the use of multi-imager sensors requires synchronization of the frame exposures between the individual imagers, a capability that has only recently been commercially available. Many widely-available imagers also use video compression to reduce both transmission bandwidth and data storage requirements. The choice of image compression, including inter- or intra-frame designs, may inhibit the recovery of the blood volume pulse signal depending on the form of the compression algorithm. Commercially-available solutions for adopting multi-imager synchronization and inter- versus- intra-frame compression were tested on stationary, seated participants while ground-truth physiological signals were simultaneously collected for comparison. The within-subjects design included analyses for pulse rate and several quantifications of pulse rate variability. Observable differences in the quality of the recovered vital sign measurements as related to the use of multiple imagers and varied video compression approaches are important for early adopters to consider.

10072-25, Session 6

Assessing photoplethysmographic imaging performance beyond facial perfusion analysis

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Photoplethysmographic imaging (PPGI) systems are relatively new non-contact biophotonic diffuse reflectance systems able to assess arterial pulsations through transient changes in light-tissue interaction. Many PPGI studies have focused on extracting heart rate from the face or hand. Though PPGI systems can be used for widefield imaging of any anatomical area, whole-body investigations are lacking. Here, using a novel PPGI system, coded hemodynamic imaging (CHI), we explored and analyzed the pulsatility at major arterial locations across the whole body, including the neck (carotid artery), arm/wrist (brachial, radial and ulner arteries), and leg/feet (popliteal and tibial arteries). CHI was positioned 1.5 m from the participant, and diffuse reflectance from a broadband tungsten-halogen illumination was filtered using 850-1000 nm bandpass filter for deep tissue penetration. Images were acquired over a highly varying 24-participant sample (11/13 female/male, age 28.7 +/- 12.4 years, BMI 25.5 +/- 5.2 kg/m2). B-mode ultrasound images were acquired to validate observations through planar arterial characteristics.

10072-26, Session 6

A multispectral testbed for cardiovascular sensing using imaging photoplethysmography

Ethan B. Blackford, Ball Aerospace & Technologies Corp. (United States); Justin R. Estepp, Air Force Research Lab. (United States)

Imaging photoplethysmography uses image sensors to measure changes in light absorption resulting from skin microvascular blood volume pulsations throughout the cardiac cycle. Imaging photoplethysmography has been demonstrated as an effective, non-contact means of assessing pulse rate, pulse rate variability, and respiration rate. Other potential uses include measuring spatial blood perfusion, oxygenation, and flow dynamics. Herein we demonstrate the development of a multispectral testbed for imaging photoplethysmography consisting of 12 monochromatic, 120fps imagers with 50nm, bandpass filters distributed from 400-750nm and contained in a 3D-printed, 4x3 grid housing mounted on a tripod positioned orthogonal to the subject. A co-located dual-CCD RGB/near-infrared imager records conventional RGB and NIR images expanding the spectral window recorded. After image registration, a multispectral image cube of the 13, partially overlapping bands is created. A spectrometer records high (spectral) resolution data from the participant's right cheek using a collimating lens attached to the measurement fiber. In addition, a spatial array of 5 RGB imagers placed at 0°, ±20° and ±40° positions with respect to the subject is employed for motion and spatial robustness. All imagers are synchronized by a hardware trigger source synchronized with a reference, physiological measurement device recording the subject's electrocardiography, fingertip and ear lobe photoplethysmography, bilateral galvanic skin response, and respiration signals. The development of the testbed and pilot data is presented. A full scale evaluation of the spectral components of the iPPG signal, optimization of iPPG SNR, and spatial perfusion and blood flow dynamics is currently underway.

10072-27, Session 6

Measurements of pulse rate and pulse rate variability using long-range imaging photoplethysmography and sunlight illumination

Ethan B. Blackford, Ball Aerospace & Technologies Corp. (United States); Justin R. Estepp, Air Force Research Lab. (United States)

Imaging photoplethysmography, a method using image sensors to record optical absorption and reflection variations caused by skin microvascular blood volume pulsations, shows promise as a non-contact cardiovascular sensing technology. Our team recently demonstrated the first long-range imaging photoplethysmography measurements at distances of 25, 50, and 100 meters from the subject. While imaging photoplethysmography time series signals recorded at a distance of 25 meters were measured with sufficient accuracy to resolve individual blood volume pulse waves and accurate instantaneous measurements of pulse rate and pulse rate variability; degraded signal quality was observed with increasing imager-to-subject distances. This decrease in signal to noise ratio prevented time-domain cardiovascular measurements from being obtained; windowed, frequency-domain measurements were still possible. The degradation in signal quality was hypothesized to be largely attributable to the inadequate light return delivered to the image sensor with increasing lens focal lengths and artificial, indoor lighting. To test this hypothesis, a follow-up evaluation with 25 participants was conducted outdoors with ~15-50 times stronger sunlight illumination. Video was recorded with enthusiast-grade, mirrorless cameras equipped with ultra-telephoto lenses mounted on tripods and positioned at distances of 25, 50, 100, and 150 meters. The brighter subject illumination allowed high-definition video recordings at increased frame rates of 60fps, shorter exposure times, and lower ISO settings, leading to higher quality image formation than the previous indoor evaluation. Simultaneous reference physiological measurements of electrocardiography, fingertip photoplethysmography, galvanic skin response, and respiration were recorded for comparison with measurements derived from long-range imaging photoplethysmography.

10072-28, Session 7

Optical monitoring of spinal cord hemodynamics

Babak Shadgan, Femke Streijger, Brian Kwon, The Univ. of British Columbia (Canada)

Introduction: Spinal cord (SC) ischemia and hypoxia are important contributors to secondary damage after traumatic spinal cord injury (SCI). To mitigate these secondary processes and improve neurologic outcome, current clinical practice guidelines recommend aggressive maintenance of spinal cord perfusion and oxygenation. Such hemodynamic management, however, is currently carried out without any real-time measures of spinal cord perfusion and oxygenation. Such information would be extremely valuable to clinicians managing such patients in the intensive care setting. This study examined the feasibility and sensitivity of a custom-made near infrared spectroscopy (NIRS) sensor to monitor SC hemodynamics and oxygenation in a pig model of spinal cord injury.

Methods: Three anesthetized Yucatan mini-pigs were studied using a NIRS system with a miniaturized optical sensor applied directly on the surgically exposed SC at T9 during a set of systemic physiological manipulations including ventilatory hypoxia and altering mean arterial pressure (MAP). Three intraparenchymal probes were inserted through the dura at T11 to invasively monitor SC oxygenation, blood flow and pressure.

Results: Non-invasive NIRS monitoring reflected changes in intraparenchymal SC oxygenation and hemodynamics in response to ventilatory-induced hypoxia and changes in MAP. The changes in intraparenchymal oxygen level and blood flow were simultaneously reflected in the changes in NIRS oxygenated and deoxygenated hemoglobin concentrations.

Conclusions: This pilot study indicates that a novel miniaturized NIRS sensor has the potential to monitor SC hemodynamics and oxygenation in real time. Further development of this method may offer new options for improved SCI care.

10072-29, Session 7

In vivo preclinical verification of a multimodal diffuse reflectance and correlation spectroscopy system for sensing tissue perfusion

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Reconstruction of complex patient defects secondary to trauma or ablative surgery is accomplished with microvascular free grafts harvested with their vascular supply i.e., artery and vein. Graft placement at the reconstruction site requires the anastomosis of the donor vessels to vessels at the recipient site to allow continued blood flow to the transferred tissue. Impeded blood flow to the grafts secondary to arterial or venous occlusion can rapidly result in graft failures. Thus, the ability to detect changes in blood perfusion in free flaps is critical. A clinically-compatible device capable of detecting full and partial occlusion in tissue flaps could serve to alert surgeons when a graft requires intervention in order to restore vascular perfusion.

Here, we report progress on pre-clinical testing of a compact, multimodal, fiber-based diffuse correlation and reflectance spectroscopy (DCS-DRS) system designed to quantitatively monitor blood perfusion in surgically-grafted free flaps in a porcine model (n = 4). We describe the in vivo protocol, which included acquiring optical measurements at three different tissue sites (the artery, muscle, and skin in the porcine flaps) as the flap's blood supply was slowly altered. Technology including hardware and software, data acquisition and analysis, and comparisons to clinical gold standards in perfusion monitoring is discussed. In addition, we compare data acquired during artificial vasocompression to that acquired during spontaneous venous congestion. Device sensitivity to incremental changes in blood flow will be quantified and the prospects for continuous perfusion monitoring in future clinical translational studies is discussed.

10072-30, Session 7

Respiratory muscle hemodynamic and metabolic adaptations to 16 weeks of training in varsity soccer players: near-infrared spectroscopy measurements during lung function tests

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The purpose of this study was to test the hypothesis that mobile, wireless near-infrared spectroscopy (NIRS) instruments can be used during standard lung function tests to measure adaptations in respiratory muscle metabolism over weeks to months. In eight varsity soccer players at 0 weeks and after 16 weeks of routine training, commercially available mobile, wireless NIRS instruments were used to measure oxygenation and hemodynamics in the sternocleidomastoid (SCM, accessory inspiration muscle). During maximal expiratory pressure (MEP) and forced vital capacity (FVC) maneuvers we determined peak or antipeak changes relative to baseline in oxygenation and hemodynamics: ?%Sat (muscle oxygen saturation), ?tHb (total hemoglobin), ?O2Hb (oxygenated hemoglobin),

and ?HHb (deoxygenated hemoglobin). Subjects reported that the average training load was ~13.3 h/week during the 16 study weeks, compared to ~10.4 h/week during 12 prior weeks. After 16 weeks of training compared to 0 weeks we found statistically significant increases in SCM ?%Sat (57.7%), ?tHb (55.3%), and ?O2Hb (56.7%) during MEP maneuvers, and in SCM ?%Sat (64.8%), ?tHb (29.4%), and ?O2Hb (51.6%) during FVC maneuvers. Our data provide preliminary evidence that NIRS measurements during standard lung function tests are sufficiently sensitive to detect improvements or declines in respiratory muscle metabolism over periods of weeks to months due to training, disease, and rehabilitation exercise.

10072-31, Session 7

Monte Carlo modeling of spatially complex wrist tissue for the optimization of optical pulse oximeters

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Monte Carlo modeling of photon propagation has been used in the examination of particular areas of the body to further enhance the understanding of light propagation through tissue. A multi-domain Monte Carlo simulation was used to accurately model propagation of light through the wrist. Specifically, a Monte Carlo simulation program was prototyped using Matlab and then implemented in C++ for increased speed. As an improvement upon modeling tissue as multilayer stacks of tissue sections, generation of different tissue domains, such as muscle, vasculature, and bone, was performed in Solidworks, where each domain was saved as a separate (.stl) file that was read into the program. The light source was altered to give considerations to both viewing angle of the simulated LED as well as the nominal diameter of the source. Results generated were compared to those generated by the multilayer stack model for similar tissue properties and spatial domains, as well as to in-vitro phantom studies of the simulated tissue. These more accurate models can be used to better guide the design of these optical measurement devices.

10072-32, Session 7

Simulation of diffuse reflectance for characterisation of particle suspensions

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Spectroscopy is commonly used to obtain the composition of a substance; however, for samples with a high weight percent of solid, such as, blood or tissue, absorption or transmission spectroscopy is no longer effective, due to the multiple light scattering effect introduced by the particulates. In these systems, it is difficult to distinguish between the contributions of scattering and absorption to the measured spectrum. Theoretical methods are required to disentangle these two processes. Current approaches use empirical or semi-empirical methods to correct the scattering induced spectral change, or use physical theory to decouple the absorption and scattering effect. These methods are followed by multivariate analysis to build a calibration model. However, the accuracy and robustness of the model could vary greatly, also the model may not be reliable for another system.

We employ a physical theory to construct a computational model that accounts for both multiple scattering and absorption of light without requiring a calibration model. This approach is based on using Mie theory to describe single particle scattering along with a corrected diffuse approximation to provide an analytical solution to the radiative transfer equation. Using polystyrene particle suspensions as the model system, we apply our method to spatially and angularly resolved diffuse reflectance measurements for a wide range of particle radii and concentrations. We benchmark the performance against approaches which require building a calibration model.

We have found that our method provides good estimates of the optical parameters from a full NIR-vis-UV spectrum in a matter of minutes.

10072-33, Session 8

Sensor system for non-invasive optical carboxy-and methemoglobin determination

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COHb and MetHb are so called dysfunctional hemoglobin and they are not able to carry oxygen. Standard pulse oximeter can only measure two derivatives, namely oxyhemoglobin (O2Hb) and deoxyhemoglobin (RHb) but the presence of other derivative in the blood can distort the readings. High MetHb concentrations can be caused by by local anesthesia, several medications or drugs. Carbon monoxide binds to hemoglobin at the same sites as oxygen, but approximately 200 times more tightly Since COHb releases carbon monoxide slowly, less hemoglobin will be available to transport oxygen from the lungs to the rest of the body. Conversion of most Hb to COHb results in death - known medically as carboxyhemoglobinemia or carbon monoxide poisoning. Smaller amounts of COHb lead to oxygen deprivation of the body causing tiredness, dizziness, and unconsciousness. By using an artificial blood flow model the concentration of each derivate could be varied separate and the optical properties were monitored by spectrometers. Based on the results an LED based Sensor system was developed. The paper will describe a novel multi-wavelength photometric method to measure the MetHb and COHB concentration non-invasively. Hypoxia studies with human subjects and animal experiments are done to prove and demonstrate the performance of the sensor system. The results are compared to the gold standard, the blood gas measurement (BGA).

10072-34, Session 8

Analyte detection in complex samples using a biomimetic, non-spectroscopic sensing method

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Optical sensing techniques for organic and biological molecules traditionally require the collection of complete, well-defined, vibrational spectra of a sample. The vibrational absorption bands of the spectra are identified, and statistical methods are then utilized to determine the moieties and concentration of target analytes present in a given sample. In this work, we present a new, non-spectroscopic alternative technique to molecular vibrational sensing. This biomimetic method, based on human color vision, uses only three broad, overlapping infrared (IR) optical filters to discriminate between chemicals with similar vibrational absorption bands. Unique detection vectors are defined by the interaction of a given chemicals absorption bands with the three filter channels. Identification of the analytes present in a sample are then determined based on these detection vectors.

We present multiple studies that demonstrate the ability of this approach to clearly discriminate between molecules with similar infrared vibrational absorption bands. We show that this method has the ability to precisely identify specific analytes in the presence of potential interferents with similar infrared absorption bands in the same sample. An optical filter based sensor that operates in the mid-IR using low power components, requiring no spectral scanning has been developed using this technique, and results using this sensor are shown. This method has the potential to lead the development of small, rugged optical sensors for non-invasive diagnostics and sensing of biological fluids.

10072-35, Session 8

Multispectral and colour analysis for ubiquinone solutions and biological samples

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Nowadays colour theory is implemented deeper into analysis of materials and pigments for the purposes of spectroscopic analysis. It has got a lot of field of application into clinical diagnostics or pharmacy because of its non-destructive properties of expertise. Calculation of colour characteristics does not concede to statistical analysis of spectra in visualization of results and precision. The purpose of this paper is to find how colour of the recording samples is changing under different experimental conditions using multispectral analysis of ubiquinone solutions and tissues in vitro as samples. Ubiquinone is the wide distributed antioxidant in pharmacy and in the human body so it was chosen as a sample for investigation. The main application of this analysis is to control the quality of medical supplement but also it has got advantages to be implemented into clinical diagnostics. Multispectral analysis provides spectra recording with following calculation of the colour and eliminates metamerism which can be the issue of analysis with the colour camera. Concentrations of ubiquinone from 0.25 to 2.5 mmol/l in recording samples were used to find dependence between colour characteristics of the sample and such conditions of storage as packaging leak, lightning and etc. It was realized that dependences colour characteristics and experimental conditions exist and can be analyzed under following chemical analysis to confirm chemical changing. This technic has got prospects in a quality control because of high precision and can be used not only for ubiquinone.

10072-36, Session 8

Measuring tryptophan concentrations of aqueous solutions for cancer research using terahertz time-domain spectroscopy with metal parallel-plate waveguides

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Recent cancer research is focusing strongly on the essential amino acid Tryptophan, as it plays a crucial role in the metabolism of cancer cells. Measurement techniques to determine Tryptophan concentrations of aqueous solutions are therefore vastly important for ongoing research in this field.

Recently, Terahertz spectroscopy has illustrated its high potential to be utilized for the characterization of bio-crystals and biomolecules. We have developed a method to detect and quantify tryptophan based on the parallel-plate waveguide (PPWG) technology together with a commercially available terahertz time domain spectroscopy (TDS) system called "T-SPECTRALYZER F" providing a spectral bandwidth from 0.1 THz to 5 THz.

As Terahertz waves are strongly absorbed by water, a measurement of aqueous solutions is a challenging task. In our setup parallel-plate waveguides (PPWG) are used to detect low Tryptophan concentrations, in principle, in solution. Drop-casting the solution into the waveguide forms a dry homogeneous film after evaporation of the solvent. A spectroscopic analysis of the transmission spectrum of the waveguide allows for a determination of the tryptophan concentration as the detection limit is drastically increased by the use of waveguides.

In order to increase the detection sensitivity of this measurement technique the THz setup was encapsulated in a dry air box to reduce water vapor effects.

Here we introduce the working mechanism of "T-SPECTRALYZER F" and present the spectral evaluation procedures applied. Finally, we show the improvement of the detection sensitivity using a terahertz time-domain spectroscopy system together with PPWG technology.

10072-37, Session 8

Rapid, sensitive and reproducible method for point-of-collection screening of liquid milk for adulterants using a portable Raman spectrometer with novel optimized sample well

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Point-of-care diagnostics are of interest in the medical, security and food industry, the latter particularly for screening food adulterated for economic gain. Milk adulteration continues to be a major problem worldwide and different methods to detect fraudulent additives have been investigated for over a century. Laboratory based methods are limited in their application to point-of-collection diagnosis and also require expensive instrumentation, chemicals and skilled technicians. This has encouraged exploration of spectroscopic methods as more rapid and inexpensive alternatives. Raman spectroscopy has excellent potential for screening of milk because of the rich complexity inherent in its signals. The rapid advances in photonic technologies and fabrication methods are enabling increasingly sensitive portable mini-Raman systems to be placed on the market that are both affordable and feasible for both point-of-care and point-of-collection applications. We have developed a powerful spectroscopic method for rapidly screening liquid milk for sucrose and four nitrogen-rich adulterants (dicyandiamide (DCD), ammonium sulphate, melamine, urea), using a combined system: a small, portable Raman spectrometer with focusing fibre optic probe and optimized reflective focusing wells, simply fabricated in aluminium. The reliable sample presentation of this system enabled high reproducibility of 8% RSD (residual standard deviation) within four minutes. Limit of detection intervals for PLS calibrations ranged between

140 – 520 ppm for the four N-rich compounds and between 0.7 – 3.6 % for sucrose. The portability of the system and reliability and reproducibility of this technique opens opportunities for general, reagentless adulteration screening of biological fluids as well as milk, at point-of-collection.

10072-38, Session 8

Design of smartphone-based spectrometer to assess fresh meat color

Youngkee Jung, Purdue Univ. (United States); Euiwon Bae, Purdue university (United States)

Based on its integrated camera, new optical attachment, and inherent computing power, we provide an objective and accurate method to determine a myoglobin content in meat using a smartphone-based spectrometer. System is flexible to be used as both transmission and reflection spectrometer which mimics the conventional spectrometry used in meat science laboratories. We utilize a 3D printing technique to make an optical cradle which holds all of the optical components for light collection, collimation, dispersion, and a suitable chamber for two kinds of samples. A light which pass a sample enters a pinhole and is subsequently collimated by a convex lens. A diffraction grating spreads the wavelength over the camera's pixels to display a high resolution of spectrum. Pixel values in the smartphone image are translated to calibrate the wavelength values through three laser pointers which have different wavelength; 405, 532, 650 nm. Using an in-house app, the camera images are converted into a spectrum in the visible wavelength range based on the exterior light source. Finally, two kinds of reflection and transmission standard spectrometers are used to evaluate the performance of developed spectrometer. For that, meat samples are prepared with a collaboration of meat science lab and given as two different types. The algorithm to detect the myoglobin percentage in meat is derived from the equation of Krzywicki (1971). We expect that this technology can be adapted to any smartphone and used to conduct a field-deployable the color spectrum assay for food inspectors.

Conference 10073: Adaptive Optics and Wavefront Control for Biological Systems III

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10073-1, Session 1

Wavefront control with a multi-actuator adaptive Lens in imaging applications *(Invited Paper)*

Jacopo Mocci, CNR-IFN Padova (Italy); Michelle Cua, Sujeen Lee, Yifan Jian, Simon Fraser Univ. (Canada); Paolo Pozzi, Technische Univ. Delft (Netherlands); Martino Quintavalla, Cosmo Trestino, CNR-IFN Padova (Italy); Hans R. G. W. Verstraete, Technische Univ. Delft (Netherlands); Riccardo Muradore, Univ. degli Studi di Verona (Italy); Robert J. Zawadzki, Univ. of California, Davis (United States); Daniel J. Wahl, Simon Fraser Univ. (Canada); Michel Verhaegen, Technische Univ. Delft (Netherlands); Marinko V. Sarunic, Simon Fraser Univ. (Canada); Stefano Bonora, CNR-IFN Padova (Italy)

The use of adaptive lenses instead of deformable mirrors can simplify the implementation of an adaptive optics system. The recently introduced Multi-actuator Adaptive Lens (MAL) can be used in closed loop with a wavefront sensor to correct for time-variant wavefront aberrations. The MAL can guarantee a level of correction and a response time similar to that obtained with deformable mirrors. The adaptive lens is based on the use of piezoelectric actuators and, without any obstruction or electrodes in the clear aperture, can guarantee a fast response time, less than ~10ms. Our tests show that the MAL can be used both in combination with a wavefront sensor in a “classical” adaptive optics closed loop, or in a wavefront sensorless configuration. The latter has allowed us to design more compact and simple imaging systems for different microscopy platforms. We will show that the Multi-actuator Adaptive Lens has been successfully used for in-vivo OCT ophthalmic imaging in both mice and humans, as well as confocal and two photon microscopy. We tested and compared different optimization strategies such as coordinate search and the DONE algorithm. The results suggests that the MAL optimization can correct for eye aberrations with a pupil of 5mm or sample induced aberrations in microscopy.

10073-2, Session 1

Spherical aberration correction of adaptive lenses

Katrin Philipp, TU Dresden (Germany); Florian Lemke, Univ. Freiburg (Germany); Matthias C. Wapler, Ulrike Wallrabe, Univ. of Freiburg (Germany); Nektarios Koukourakis, Jürgen W. Czarske, TU Dresden (Germany)

Adaptive lenses have become a suitable tool for axial scanning in imaging systems. They offer advantages like fast tuning speeds in the kHz range and motionless axial scanning which is crucial in particular for microscopic techniques. A remaining disadvantage of axial scanning with adaptive lenses is the introduction of aberrations to the wavefront by the tuning process. Lately, there have been some efforts to produce lenses with more degrees of freedom, aiming to tune specific aberrations in addition to defocus. The most promising aspect for the application in microscopes is the correction of the dominating spherical aberrations. Spherical aberrations are induced by the optical components, immersion fluids and mainly by the specimen itself. Aberration correction in confocal microscopes has successfully been conducted with deformable mirrors. However, deformable mirrors are bulky and expensive and hence might not be the best solution for a broad range of applications. The use of adaptive lenses with aberration correction is a promising approach towards high-resolution, high-speed microscopy, e.g. of zebrafish embryos. In this contribution, we present and characterize a novel adaptive lens with aberration correction first described in [M. Wapler et al., doi:org/10.1109/ISOT.2014.39]. We use digital holography to characterize the change of the Zernike polynomials as a function of the actuation voltages of the adaptive lens and further present iterative methods to adjust the lens for a targeted spherical parameter. We discuss the potential of the application of these lenses in confocal microscopy for aberration correction.

10073-3, Session 1

Wavefront shaping with an electrowetting liquid lens using surface harmonics

Matthias Strauch, Sander Konijnenberg, Yifeng Shao, H. Paul Urbach, Technische Univ. Delft (Netherlands)

Liquid lenses are used to correct for low order wavefront aberrations. Electrowetting liquid lenses can nowadays control defocus and astigmatism effectively, so they start being used for ophthalmology applications. To increase the performance and applicability, we introduce a new driving mechanism to create, detect and correct higher order aberrations using standing waves on the liquid interface.

The speed of a liquid lens is in general limited, because the liquid surface cannot follow fast voltage changes, while providing a spherical surface. Surface waves are created instead and with them undesired aberrations. We try to control those surface waves to turn them into an effective wavefront shaping tool.

We introduce a model, which treats the liquid lens as a circular vibrating membrane with adjusted boundary conditions. Similar to tunable acoustic gradient (TAG) lenses, the nature of the surface modes are predicted to be Bessel functions. Since Bessel functions are a full set of orthogonal basis functions any surface can be created as a linear combination of different Bessel functions.

The model was investigated experimentally in two setups. First the point spread functions were studied and compared to a simulation of the intensity distribution created by Fresnel propagated Bessel surfaces. Second the wavefronts were measured directly using a spatial light modulator. The surface resonance frequencies confirm the predictions made by the model as well as the wavefront measurements. By superposition of known surface modes, it is possible to create new surface shapes, which can be used to simulate and measure the human eye.

10073-4, Session 2

Generation of light-sheet at the end of multimode fibre

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Light-sheet fluorescence microscopy is quickly becoming one of the cornerstone imaging techniques in biology as it provides rapid, three-dimensional sectioning of specimens at minimal levels of phototoxicity. It is very appealing to bring this unique combination of imaging properties into an endoscopic setting and be able to perform optical sectioning deep in tissues.

Current endoscopic approaches for delivery of light-sheet illumination are based on single-mode optical fibre terminated by cylindrical gradient index lens. Such configuration generates a light-sheet plane that is axially fixed and a mechanical movement of either the sample or the endoscope is required to acquire three-dimensional information about the sample. Furthermore, the axial resolution of this technique is limited to 5um.

The delivery of the light-sheet through the multimode fibre provides better axial resolution limited only by its numerical aperture, the light-sheet is scanned holographically without any mechanical movement, and multiple advanced light-sheet imaging modalities, such as Bessel and structured illumination Bessel beam, are intrinsically supported by the system due to the cylindrical symmetry of the fibre.

We discuss the holographic techniques for generation of multiple light-sheet types and demonstrate the imaging on a sample of fluorescent beads fixed in agarose gel, as well as on a biological sample of Spirobranchus Lamarcki.

10073-5, Session 2

new depths for Airy light-sheet microscopy: attenuation compensation techniques to increase imaging depths within absorbing samples

Jonathan Nylk, Miguel A. Preciado, Michael Mazilu, Kishan Dholakia, Univ. of St. Andrews (United Kingdom)

Light-sheet microscopy (LSM) is an emergent fluorescence microscopy technique showing great promise for biomedical research. LSM enables rapid, high-contrast imaging of large specimens with high spatiotemporal resolution and minimal photo-damage. When imaging large specimens, the intensity of the light-sheet reduces across the field-of-view (FOV) due to absorption. This results in an image with spatially-variant intensity, affecting quantitative measurements, and ultimately limits the penetration depth of the illumination. Some existing approaches to alleviate this issue involve illuminating the sample from multiple directions or rotating the sample. These approaches are not always practical and restrict specimen choice.

Separately, propagation-invariant light modes have been used to develop high-resolution LSM techniques as they can overcome the natural divergence of a Gaussian beam, producing a thin and uniform light-sheet over long distances. Most notably, Bessel and Airy beam-based LSM techniques have been implemented.

For propagation-invariant beams, there exists a mapping between the transverse coordinate in the pupil plane of a lens, and the axial propagation in the focal plane. Spatially-variant amplitude modulation therefore offers control of the intensity of the beam with propagation.

In this paper, we report that such amplitude modulation in the pupil plane of an Airy LSM can yield a system which counteracts absorption of the light-sheet and gives uniform intensity across the FOV with a single acquisition and without restricting specimen choice. This technique is an alternative to, and may be complimented by, wavefront correction. We demonstrate this technique through numerical simulations and experimental validation in absorbing tissue phantoms.

10073-6, Session 2

Use of light-sheet microscopy combined with wavefront coding for high-resolution 3D imaging of biological samples

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Some biological experiments demand the observation of dynamics processes in 3D with high spatiotemporal resolution. The use of wavefront coding to extend the depth-of-field (DOF) of the collection arm of a light-sheet microscope is an interesting alternative for fast 3D imaging. Under this scheme, the 3D features of the sample are captured at high volumetric rates while the light sheet is swept rapidly within the extended DOF. The DOF is extended by coding the pupil function of the imaging lens by using a custom-designed phase mask. A posterior restoration step is required to decode the information of the captured images based on the applied phase mask [1]. This hybrid optical-digital approach is known as wavefront coding (WFC). Previously, we have demonstrated this method for performing fast 3D imaging of biological samples at medium resolution [2]. In this work, we present the extension of this approach for high-resolution microscopes. Under these conditions, the effective DOF of a standard high NA objective is of a few micrometers. Here we demonstrate that by the use of WFC, we can extend the DOF more than one order of magnitude keeping the high-resolution imaging. This is demonstrated for two designed phase masks using Zebrafish and C. elegans samples.

[1] Olarte, O.E., Andilla, J., Artigas, D., and Loza-Alvarez, P., "Decoupled Illumination-Detection Microscopy. Selected Optics in Year 2105," in Optics & Photonics news 26, p. 41 (2015).

[2] Olarte, O.E., Andilla, J., Artigas, D., and Loza-Alvarez, P., "Decoupled illumination detection in light sheet microscopy for fast volumetric imaging," Optica 2(8), 702 (2015).

10073-7, Session 3

Biophotonics for imaging through complex biological systems: adaptive wavefront shaping technologies and phase retrieval reconstructions

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Despite the immense progress in biophotonics and biomedical imaging, significant challenges remain that limit high resolution imaging in the few mean free paths regime (<1mm). These challenges are associated to the diffusive nature of light propagation through biological tissue caused by the random variations of the refractive index. Improving the depth to resolution ratio requires the use of adaptive wavefront technologies and smart phase retrieval algorithms to exploit the high number of optical paths in coherent multiply scattered light. Combined with appropriately engineered or biomimetic photonic structures a significant improvement in microscopic imaging performance is achieved. Paradigms of such approaches are presented for: i) light sheet microscopy with the production of achromatic, scan-less, tailored light sheets using opaque cylindrical lenses; ii) the selection of specific spatial frequencies from the speckle patterns or the production of non-diffracting sources with appropriate spatial filtering and wavefront shaping; iii) applying phase retrieval to multi-angle light sheet microscopy imaging of opaque 3D live cancer cell spheroids to improve depth resolution of specific biomarkers.

These technologies offer for the first time the opportunity to image deep in opaque complex biological media with unprecedented resolution. The next generation of multiscale biomedical imaging systems will capitalize on the use of ultrafast wavefront shaping devices and parallel processing to achieve deep high resolution imaging in real time.

10073-8, Session 3

Transmission-matrix-based point-spread-function engineering through a complex medium

Antoine Boniface, Mickael Mounaix, Baptiste Blochet, Lab.

Kastler Brossel (France); Rafael Piestun, Univ. of Colorado Boulder (United States); Sylvain Gigan, Lab. Kastler Brossel (France)

When coherent light propagates through a disordered system, such as white paint or biological tissue, its spatial properties are mixed and the resulting transmitted field forms a speckle pattern. Although the size of a speckle grain is diffraction-limited, this complex interference figure is detrimental for all conventional imaging systems.

Recently, wavefront shaping techniques have opened a new way to perform imaging through disordered systems, using spatial light modulators. In particular, the optical transmission matrix (TM) links the input field to the output field It enables arbitrary spatial focusing of light after propagation in the medium, whose size is limited by diffraction.

We report the first formulation of a TM-based operator that enable the focusing of arbitrary point-spread-function (PSF) after propagation in the medium. We numerically compute the optical field in a virtual pupil plane by Fourier transforming the output speckle pattern. By numerically applying an arbitrary (phase and/or amplitude) mask onto this pupil, we build on this effective operator. It enables focusing of the corresponding PSF at the output of the medium.

As an example, we demonstrate the generation of Bessel beam using an amplitude annular mask, and show that the FWHM of its central peak is narrower than the size of a speckle grain. The generation of sub-diffraction pattern at the output is thus now deterministically achievable. We also excite Laguerre-Gauss beam ("donut") using a vortex masks, as well as even more complex 3D-beam profiles such as spiral PSFs, which paves the way for super-resolution imaging in turbid media.

10073-9, Session 3

Simultaneous depth mapping fluorescent microscopy based on self-bending point spread function

Seongjun Park, Institute for Basic Science (Korea, Republic of); Chan Young Lee, Ulsan National Institute of Science and Technology (Korea, Republic of); Sang-Hee Shim, Korea Univ. (Korea, Republic of); Francois AMBLARD, Institute for Basic Science (Korea, Republic of); Sung Chul Bae, Ulsan National Institute of Science and Technology (Korea, Republic of) and Institute for Basic Science (Korea, Republic of)

Airy beam has many unique properties such as non-diffracting during the propagation and self-accelerating without any external forces; it offers many applications such as optical micromanipulation of particles, plasma guidance, vacuum electron acceleration, and generation of three-dimensional optical bullets. Recently, the stochastic optical reconstruction microscopy (STORM) has been reported based on a self-bending point spread function (SB-PSF) derived from Airy beams. As Airy beams undergo lateral displacement during their propagation without significant diffraction, it permits precise three-dimensional localization of molecules over large imaging depth by mapping the axial position in the detection plane. Here, we report a fast three-dimensional fluorescent microscope based on SB-PSF derived from Airy beams with no sample scanning. We used a Gaussian-Bessel beam to illuminate an extended depth of the sample with reduced side lobe energy for the excitation. In the detection path, a polarization-insensitive spatial light modulator was placed at the Fourier plane to produce Airy beam by introducing the cubic spatial phase for the detection. Using this method, we achieved three dimensional fluorescent imaging over an extended imaging depth (6 times than that of Gaussian PSF) without z scanning. This SB-PSF based approach could be useful for instantons x-z imaging, and therefore, for fast imaging of 3D volumes without moving the focal plane.

10073-10, Session 3

Bessel beams for retinal imaging OCT

Sandra E. Balderas-Mata, Univ. de Guadalajara (Mexico)

A theoretical analysis of the advantages of using Bessel beams over the use of Gaussian beams for Fourier domain Optical Coherence Tomography for retinal imaging is presented.

10073-54, Session 3

Single and two-photon swept confocally aligned planar excitation (SCAPE) microscopy for high-speed 3D imaging of neural activity

Pubudu T. Galwaduge, Hang Yu, Venkatakaushik Voleti, Kripa Patel, Wenzi Li, Elizabeth M. Hillman, Columbia Univ. (United States)

Light-sheet microscopy has historically relied on the use of 2 or more orthogonal objective lenses. The need to physically synchronize scanning of the light sheet and detection plane limits volumetric imaging rates, while the imaging geometry restricts sample shape and size and requires complex mounting of specimens that impairs observations of free behavior. SCAPE microscopy instead illuminates the sample using an oblique light sheet, detecting fluorescent light through the same objective. A galvanometer scanner sweeps this sheet through the sample, while also de-scanning returning light resulting in an oblique, stationary image plane that is always aligned with the illumination sheet in the sample. Image rotation relays this plane onto a high-speed camera. A 3D volume is formed for a single sweep of the galvanometer mirror, such that imaging at 40 VPS requires scanning at only 40 lines per second with no other moving parts in the system.

SCAPE has been demonstrated on a wide range of living samples, including imaging structure and function in freely crawling Drosophila larva, the whole adult Drosophila brain during behavior, zebrafish brain and heart, and the awake, behaving mouse brain. In ongoing work, we are combining SCAPE microscopy with two-photon excitation with the goal of achieving superior depth penetration and lower background excitation compared to our current implementation with 488 nm excitation. We will report on strategies that make use of lasers with adjustable pulse energies and repetition rates to enable high-speed light-sheet excitation.

10073-11, Session 4

Focusing light through or inside scattering media with millisecond digital optical phase conjugation

Yan Liu, Cheng Ma, Yuecheng Shen, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Optical phase conjugation based wavefront shaping techniques are being actively developed to focus light through or inside scattering media such as biological tissue, and they promise to revolutionize optical imaging, manipulation, and therapy. The speed of digital optical phase conjugation (DOPC) has been limited by the low speeds of cameras and spatial light modulators (SLMs), preventing DOPC from being applied to thick living tissue. Recently, a fast DOPC system was developed based on a single-shot wavefront measurement method, a field programmable gate array (FPGA) for data processing, and a digital micromirror device (DMD) for fast modulation. However, this system has the following limitations. First, the reported single-shot wavefront measurement method does not work when our goal is to focus light inside, instead of through, scattering media. Second, the DMD performed binary amplitude modulation, which resulted in a lower focusing contrast compared with that of phase modulations. Third,

the optical fluence threshold causing DMDs to malfunction under pulsed laser illumination is lower than that of liquid crystal based SLMs, and the system alignment is significantly complicated by the oblique reflection angle of the DMD. Here, we developed a simple but high-speed DOPC system using a ferroelectric liquid crystal based SLM (512 ? 512 pixels), and focused light through three diffusers within 4.7 ms. Using focused-ultrasound-guided DOPC along with a double exposure scheme, we focused light inside a scattering medium containing two diffusers within 7.7 ms, thus achieving the fastest digital time-reversed ultrasonically encoded (TRUE) optical focusing to date.

10073-12, Session 4

Focusing light through biological tissue and tissue-mimicking phantoms up to 9.6 centimeters in thickness with digital optical phase conjugation

Yuecheng Shen, Yan Liu, Cheng Ma, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Focusing light through or inside scattering media such as biological tissue is critical in many applications, such as high-resolution fluorescence imaging, photodynamic/photothermal therapy, non-invasive optogenetics, laser surgery, and optical tweezers. However, light scattering caused by the microscopic refractive index inhomogeneities inherent in biological tissue prohibits optical focusing beyond ~1 mm in depth. To break this optical diffusion limit, wavefront shaping techniques such as optical phase conjugation (OPC) have been developed to focus light through or within biological tissue. However, due to practical considerations such as an insufficiently strong light signal, a short speckle correlation time, and an inadequate laser coherence length, the thicknesses of samples used in previous studies were limited to only a few millimeters or several transport mean free paths, which is still relatively shallow for many pre-clinical and clinical applications. Here, by using a laser with a long coherence length and an optimized digital OPC system that can safely deliver more light power, we focused 532 nm light through tissue-mimicking phantoms up to 9.6 cm thick, as well as through ex vivo chicken breast tissue up to 2.5 cm thick. Our results demonstrate that OPC can be achieved even when photons have experienced on average 1000 scattering events. The demonstrated penetration of nearly 10 cm (~100 transport mean free paths) has never been achieved before by any optical focusing or imaging technique, and it shows the promise of OPC for deep tissue non-invasive optical imaging, manipulation, and therapy.

10073-13, Session 4

One-wave phase conjugation mirror using a single-mode reflector

KyeoReh Lee, Junsung Lee, KAIST (Korea, Republic of); Jung-Hoon Park, KAIST (Korea, Republic of) and Ulsan National Institute of Science and Technology (Korea, Republic of); Ji Ho Park, YongKeun Park, KAIST (Korea, Republic of)

Phase conjugation mirror (PCM) is an optical device that reflects the phase conjugated form of incident light. The synonym of the PCM is 'time-reversal mirror' because the phase conjugated light will exactly retrace back the incident optical path regardless of the degree of scattering. This intriguing phenomenon have been extensively and steadily studied from the middle of 20th century. However, yet several PCM techniques have been proposed such as nonlinear PCM, and digital PCM; the techniques should be assisted by the 'reference arm', which is unnecessary for an ordinary mirror, or an ideal concept PCM. Further, the interferometric system make system extremely sensitive to the ambient noises that also severely discourage the practical implementations.

Here, we propose an 'one-wave' phase conjugation mirror that requires only incident light like as an ordinary mirror. We pinpointed that counter propagating lights in the single-mode waveguide is always phase-conjugation of each other. Therefore, after coupling arbitrary incident light into the single-mode waveguide via wavefront shaping, its time-reversed light can be simply amplified by coupling the high-power source on the other side of the waveguide. Using the proposed idea, we experimentally demonstrate the time-reversal of severely diffused light from the phantom tissue, chicken breasts tissue and multimode fiber. Since this concept only requires the incident light (one-wave) without reference light, the system practically does not affect by the ambient noises, and is also conceptually closer to the ideal imagination of time-reversal mirror.

10073-14, Session 4

Focusing light in deep tissue with novel guidestar assisted optical phase conjugation

Haowen Ruan, Joshua Brake, Mooseok Jang, Changhuei Yang, California Institute of Technology (United States)

Optical scattering of biological tissue limits the working depth of conventional biomedical optics, which relies on the detection of ballistic photons. Recent developed optical phase conjugation (OPC) technique breaks through this depth limit by harnessing the scattered photons and shaping an optical wavefront that can "undo" the optical scattering. The OPC system measures the complex light field exiting the tissue and reconstructs a phase conjugated copy of the measured wavefront, which propagates in the reversed direction to the source of the light. To focus light inside a scattering medium, an embedded light source or "guidestar" is often required. Therefore, developing guidestar mechanisms plays an important role in advancing the OPC technique for deep tissue optical focusing and imaging. In addition to having strong optical modulation efficiency and compact size, a favorable guidestar for biomedical applications should also have good biocompatibility, fast response time, and be noninvasive or require only minimally invasive procedure. While a number of guidestar mechanisms have been developed and showed promising for various biomedical applications, they all have their own limitations. We have been developing new guidestars and tailoring them to meet the need for biomedical imaging and therapies. We are going to present our recent progress in novel guidestar development, compare them with established guidestar mechanisms, and discuss their potential in biomedical applications.

10073-15, Session 5

Light in flight photography and applications *(Invited Paper)*

Daniele Faccio, Heriot-Watt Univ. (United Kingdom)

The first successful attempts (Abramson) at capturing light in flight relied on the holographic interference between the ``object'' beam scattered from a screen and a short reference pulse propagating at an angle, acting as an ultrafast shutter cite{egg}. This interference pattern was recorded on a photographic plate or film and allowed the visualisation of light as it propagated through complex environments with unprecedented temporal and spatial resolution. More recently, advances in ultrafast camera technology and in particular the use of picosecond resolution streak cameras allowed the direct digital recording of a light pulse propagating through a plastic bottle (Rasker at el.). This represented a remarkable step forward as it provided the first ever video recording (in the traditional sense with which one intends a video, i.e. something that can be played back directly on a screen and saved in digital format) of a pulse of light in flight.

We will discuss a different technology that is based on an imaging camera with a pixel array in which each individual pixel is a single photon avalanche diode (SPAD). SPADs offer both sensitivity to single photons and

picosecond temporal resolution of the photon arrival time (with respect to a trigger event). When adding imaging capability, SPAD arrays can deliver videos of light pulse propagating in free space, without the need for a scattering medium or diffuser as in all previous work (Gariepy et al). This capability can then be harnessed for a variety of applications. We will discuss the details of SPAD camera detection of moving objects (e.g. human beings) that are hidden from view and then conclude with a discussion of future perspectives in the field of bio-imaging.

10073-16, Session 5

Enhanced resolution and field of view through a gradient index rod by optimal static aberration compensation

Dirk E. Boonzajer Flaes, Stefan M. Witte, Johannes F. de Boer, Vrije Univ. Amsterdam (Netherlands); Tomá? Cizmár, Univ. of Dundee (United Kingdom)

Gradient index (GRIN) rods are widely used for endoscopic imaging. Although they are larger than endoscopes based on multimode fibers, they do not require knowledge of the transmission matrix to be useful for imaging, they are easier to steer and the rod's optical performance is much more resilient to bending.

However, GRIN rods are optimized for on-axis imaging, and imaging performance degrades towards the edges of the field of view. This deterioration is dependent on the length of the rod in pitch lengths. As a result, applications are limited to either a small field of view, a low resolution, or a short rod.

We investigated if a single optimal plane exists where imaging aberrations across the entire field of view can be compensated using a static mask. This would allow for a high-speed imaging system where a fast scanner is combined with a static aberration compensating device. From a theoretical perspective, the location of this compensation plane is not obvious, nor if a single plane suffices to compensate imaging aberrations induced by the rod.

We measured the transmission matrix of a large diameter, high NA GRIN rod, and numerically demonstrate that a single optimal location for wavefront correction exists, and that there is an associated optimal field. This matches our model based on light propagation through a multimode GRIN rod. The model could be used to compute the optimal rod length when the compensation plane cannot be chosen freely, such as in a commercial scanning microscope.

10073-17, Session 5

Double-pass imaging through scattering *(Invited Paper)*

Enrique Tajahuerce, Institute of New Imaging Technologies, Univ. Jaume I (Spain); Pedro Andrés Bou, Univ. de València (Spain); Pablo Artal, Lab. de Óptica Univ. de Murcia (Spain); Jesús Lancis, Institute of New Imaging Technologies, Univ. Jaume I (Spain)

In the last years, single-pixel imaging (SPI) was established as a suitable tool for non-invasive imaging of an absorbing object completely embedded in an inhomogeneous medium. One of the main characteristics of the technique is that it uses very simple sensors (bucket detectors such as photodiodes or photomultiplier tubes) combined with structured illumination and mathematical algorithms to recover the image. This reduction in complexity of the sensing device gives these systems the opportunity to obtain images at shallow depth overcoming the scattering problem. Nonetheless, some challenges, such as the need for improved signal-to-noise or the frame rate, remain to be tackled before extensive use in practical systems. Also, for intact or live optically thick tissues, epi-detection is commonly used, while present implementations of SPI are limited to transillumination geometries. In this work we present new features and some recent advances in SPI that involve either the use of computationally efficient algorithms for adaptive sensing or a balanced detection mechanism. Additionally, SPI has been adapted to handle reflected light to create a double pass optical system. Such developments represent a significant step towards the use of SPI in more realistic scenarios, especially in biophotonics applications. In particular, we show the design of a single-pixel ophtalmoscope as a novel way of imaging the retina in real time.

10073-18, Session 5

Computational Imaging for Inverse Scattering *(Invited Paper)*

Ioannis Gkioulekas, Harvard Univ. (United States)

We approach inverse scattering as an appearance matching problem: given a set of measurements at the boundary of a material, we search for the scattering parameters which, when used to synthesize new images, minimize the difference from the captured ones. We present several contributions for making this extremely multi-path and high-dimensional inverse problem tractable.

First, we introduce a computational framework that combines stochastic optimization and Monte Carlo rendering, to efficiently solve the appearance matching problem. Our framework allows inverting the light transport process in a broad range of scattering materials, without having to rely on common approximations such as single scattering and diffusion. Additionally, it accommodates rich, high-dimensional material representations, such as those required for general heterogeneous volumes.

Second, we present an acquisition system for capturing rich measurements of scattering materials, to be used as input to the optimization algorithm. Our system extends the optical coherence tomography framework, to separately measure photons based on characteristics of the paths they travel inside the material, such as length and endpoints. The use of interferometry enables us to collect such decomposed measurements at the micron-scale resolutions necessary for turbid materials.

Finally, we show how to combine these two components into a computational imaging pipeline that enables us to acquire scattering parameters for many types of materials, from everyday chemicals like waxes and soaps, to industrial coatings, which could not be accurately measured before.

10073-29, Session 5

Comparative study of six optimization methods for iterative wavefront shaping

Parsa Omidi, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States); Zahra Fayyaz, Sharif Univ. of Technology (Iran, Islamic Republic of); Nafiseh Mohammadian, Ali Hariri, Wayne State Univ. (United States)

Light propagation in turbid media, such as biological tissues, experience scattering due to inhomogeneous distribution of refractive indices. Control of light scattering is important for focusing the light or imaging through biological tissues. By spatially shaping the wave-front of the incident beam, using spatial light modulator (SLM), the scattered light can be focused to a point. Iterative optimization is a popular way of obtaining the most optimum phase map on the SLM. In this study, we implement six optimization algorithms including random search, continuous sequential, simulated annealing, particle swarm optimization, genetic algorithm, and pattern search to obtain the optimum phase map of the SLM. The main characteristics of the algorithms such as convergence time, improvement ratio and performance are compared and discussed.

10073-20, Session 6

In-vivo scattering compensation by focus scanning holographic aberration probing (F-SHARP) *(Invited Paper)*

Ioannis N. Papadopoulos, Charité Universitätsmedizin Berlin (Germany); Jean-Sébastien Jouhanneau, James Poulet, Max-Delbrück-Ctr. für Molekulare Medizin Berlin-Buch (Germany); Benjamin Judkewitz, Bioimaging and Neurophotonics Lab. Charité Universitätsmedizin Berlin (Germany)

Optical microscopy is an indispensable tool for researchers, allowing them to closely investigate different organisms, revealing new features and phenomena in biomedical research. Although very useful, conventional imaging techniques that rely only on ballistic, unaffected photons to form images inside inhomogeneous media, like biological tissue, are eventually limited up to the diffusion regime of optical propagation where scattering becomes dominant and no ballistic light can be detected.

Adaptive optics and nonlinear optimization methods that rely on so called guide stars have been employed to overcome this problem and image deeper inside biological tissue. These techniques attempt to recover the optimal wavefront that will enhance the image quality or that will render a focus spot inside the scattering biological tissue. In order to achieve that, they have to iterate through each correction mode (e.g. each pixel on a wavefront shaper) thus trading off measurement time with wavefront resolution. Here we present a new turbidity suppression approach, termed Focus Scanning Holographic Aberration Probing (F-SHARP or F?) that allows us to directly measure the amplitude and phase of the scattered light distribution at the focal plane (scattered E-field PSF). Knowledge of the E-field enables rapid correction of both aberration and scattering with a high resolution. We demonstrate the power of F-SHARP by correcting for aberration and scattering and imaging neuronal structures through the larval zebrafish and mouse brain and through thinned mouse skull in vivo.

10073-21, Session 6

Correlation effects in focused transmission through disordered media

Chia Wei Hsu, Seng Fatt Liew, Yale Univ. (United States); Arthur Goetschy, ESPCI ParisTech (France); Hui Cao, A. Douglas Stone, Yale Univ. (United States)

By controlling the many degrees of freedom in the incident wavefront, one can manipulate wave propagation in complex structures. Such wavefront-shaping methods have been used extensively for controlling light transmitted into wavelength-scale regions (speckles), a property that is insensitive to correlations in the speckle pattern. Extending coherent control to larger regions is of great interest both scientifically and for applications such as optical communications, photothermal therapy, and the imaging of large objects within or behind a diffusive medium. However, waves diffusing through a disordered medium are known to exhibit non-local intensity correlations, and their effect on coherent control has not been fully understood. Here, we demonstrate the effects of correlations with wavefront-shaping experiments on a scattering sample of zinc oxide microparticles. Long-range correlations substantially increase the dynamic range of coherent control over light transmitted onto larger target regions, far beyond what would be achievable if correlations were negligible. This and other effects of correlations emerge when the number of speckles targeted, M2, exceeds the dimensionless conductance g. Using a filtered random matrix ensemble appropriate for describing coherent diffusion and the lateral spreading in an open geometry, we show analytically that M2/g appears as the controlling parameter in universal scaling laws for several statistical properties of interest---predictions that we quantitatively confirm with experimental data. Our work elucidates the roles of speckle correlations and provides a general theoretical framework for modeling open systems in wavefront-shaping experiments.

10073-22, Session 6

Control of energy density inside turbid medium

Raktim Sarma, Yale Univ. (United States); Alexey Yamilov, Sasha Petrenko, Missouri Univ. of Science and Technology (United States); Yaron Bromberg, Hui Cao, Yale Univ. (United States)

Recent breakthroughs in optical wavefront engineering have opened the possibility of controlling light intensity distribution inside highly scattering medium, but their success is limited by the open geometry of the sample and the difficulty of covering all input modes. Here we demonstrate experimentally an efficient control of energy density distribution inside a strong scattering medium. Instead of the open slab geometry, we fabricate a silicon waveguide that contains scatterers and has reflecting sidewalls. The intensity distribution inside the 2D waveguide is probed from the third dimension. With a careful design of the on-chip coupling waveguide, we can access all the input modes. Such unprecedented control of incident wavefront leads to 10 times enhancement of the total transmission or 50 times suppression. A direct probe of light intensity distribution inside the disordered structure reveals that selective excitation of open channels leads to an energy buildup deep inside the scattering medium, while the excitation of closed channels greatly reduces the penetration depth. Compared to the linear decay for random input fields, the optimized wavefront can produce an intensity profile that is either peaked near the center of the waveguide or decay exponentially with depth. The total energy stored inside the waveguide is increased 3.7 times or decreased 2 times. Since the energy density dictates light-matter interactions inside a scattering system, our results demonstrate the possibility of tailoring optical excitations as well as linear and nonlinear optical processes inside the turbid medium in an on-chip platform.

10073-23, Session 6

Ultra-high enhancement of focusing through scattering media

Hyeonseung Yu, KyeoReh Lee, YongKeun Park, KAIST (Korea, Republic of)

Developing an efficient strategy for light focusing through scattering media is an important topic in the study of multiple light scattering. The enhancement factor of the light focusing, defined as the ratio between the optimized intensity and the background intensity is proportional to the number of controlling modes in a spatial light modulator (SLM). The demonstrated enhancement factors in previous studies are typically less than 1,000 due to several limiting factors, such as the slow refresh rate of a LCoS SLM, long optimization time, and lack of an efficient algorithm for high controlling modes. A digital micro-mirror device is an amplitude modulator, which is recently widely used for fast optimization through dynamic biological tissues. The fast frame rate of the DMD up to 16 kHz can also be exploited for increasing the number of controlling modes. However, the manipulation of large pattern data and efficient calculation of the optimized pattern remained as an issue.

In this work, we demonstrate the enhancement factor more than 100,000 in focusing through scattering media by using 1 Mega controlling modes of a DMD. Through careful synchronization between a DMD, a photo-detector and an additional computer for parallel optimization, we achieved the unprecedented enhancement factor with 75 mins of the optimization time. We discuss the design principles of the system and the possible applications of the enhanced light focusing.

10073-24, Session 6

Deterministic light focusing in space and time through multiple scattering media with a Time-Resolved Transmission Matrix approach

Mickael Mounaix, Hugo Defienne, Sylvain Gigan, Lab. Kastler Brossel (France)

When an ultrashort pulse of light propagates in a scattering medium, its spatial and temporal properties get mixed and distorted because of the scattering process. Spatially, the output pattern is the result of the multiple interference between the scattered photons. Temporally, light gets stretched within the medium due to its characteristic confinement time, thus the output pulse is broadened in the time domain. Nonetheless, as the scattering process is linear and deterministic, the spatio-temporal profile of light at the output can be controlled by shaping the input light using a single spatial light modulator (SLM).

We report the first experimental measurement of the Time-Resolved Transmission Matrix of a multiple scattering medium using a coherent time-gated detection system. This operator contains the relationship between the input field, controllable with a SLM, and the output field accessible with a CCD camera for a given arrival time of photons at the output of medium. The delay line of the time-gated detection system sets the arrival time at will within the time of flight distribution of photons of the output pulse.

We exploit this time-resolved matrix to achieve spatio-temporal focusing of the output pulse at any arbitrary space and time position. The pulse is recompressed in time to its original Fourier-limited temporal width and spatially to the diffraction-limited size defined by the speckle grain size. We also generate more sophisticated spatio-temporal profiles such as pump-probe like pulse, thus opening interesting perspectives in coherent control, light-matter interaction and imaging in disordered media.

10073-25, Session 6

Focusing through biological tissues using fast wavefront shaping

Baptiste Blochet, Lab. Kastler Brossel (France) and Institut de Biologie de l'École Normale Supérieure (France); Laurent Bourdieu, Ecole Normale Supérieure (France); Sylvain Gigan, Lab. Kastler Brossel (France)

The propagation of light in biological tissues is rapidly dominated by multiple scattering: ballistic light is exponentially attenuated, which limits the penetration depth of conventional microscopy techniques. For coherent light, the recombination of the different scattered paths creates a complex interference: speckle. Recently, different wavefront shaping techniques have been developed to coherently manipulate the speckle. It opens the possibility to focus light through complex media and ultimately to image in them, provided however that the medium can be considered as stationary.

We have studied the possibility to focus in and through time-varying biological tissues. Their intrinsic temporal dynamics creates a fast decorrelation of the speckle pattern. Therefore, focusing through biological tissues requires fast wavefront shaping devices, sensors and algorithms. We have investigated the use of a MEMS-based spatial light modulator (SLM) and a fast photodetector, combined with FPGA electronics to implement a closed-loop optimization. Our optimization process is just limited by the temporal dynamics of the SLM (200μs) and the computation time (45μs), thus corresponding to a rate of 4 kHz. To our knowledge, it's the fastest closed loop optimization using phase modulators.

We have studied the focusing through colloidal solutions of TiO2 particles in glycerol, allowing tunable temporal stability, and scattering properties similar to biological tissues. We have shown that our set-up fulfills the required characteristics (speed, enhancement) to focus through biological tissues. We are currently investigating the focusing through acute rat brain slices and the memory effect in dynamic scattering media.

10073-19, Session 7

Optical imaging through dynamic turbid media using the Fourier-domain shower-curtain effect *(Invited Paper)*

Eitan Edrei, Univ. of Maryland (United States)

The shower-curtain effect is a familiar phenomenon, routinely observed in our everyday life: an object placed behind a scattering layer appears blurred but if the object is attached to the scattering layer it can be clearly resolved. The optical system we developed takes advantage of the shower-curtain effect properties and generalizes them to achieve high-resolution imaging of objects placed at a nearly arbitrary distance behind the scattering medium. The imaging procedure is based on retrieving the object Fourier transform from the turbid medium (used as the shower-curtain) through a correlography technique based on speckle illumination. Illuminating the object with a speckle pattern rather than a coherent beam, we show that the correlography principles can be effectively applied in the near field. While the far-field condition is usually known as z>(2D^2)? (D, size of the object; ? wavelength); by tuning the spatial coherence of the illumination beam, as one can do with speckle illumination, the "far-field" condition can be written as z>(2DRc)? where Rc is the correlation radius of the speckle pattern.

Using our method we present high-resolution imaging of objects hidden behind millimeter-thick tissue or dense lens cataracts, and demonstrate our imaging technique to be insensitive to rapid medium movements (>5 m?s) beyond any biologically relevant motion. Furthermore, we show this method can be extended to several contrast mechanisms and imaging configurations.

10073-26, Session 7

Resolution in two-photon infrared vision

Pablo Artal, Lab. de Óptica Univ. de Murcia (Spain); Katarzyna Komar, Nicolaus Copernicus Univ. (Poland); Adrian Gambin, Silvestre Manzanera, Univ. de Murcia (Spain); Maciej Wojtkowski, Nicolaus Copernicus Univ. (Poland)

Human subjects can detect infrared light at wavelengths over 1000 nm perceived as visible of the corresponding half wavelength. This is due to a two-photon process and requires the use of pulsed light sources well focused within the retina. We have developed an experimental system to measure, for the first time, the visual resolution of the eye when is stimulated with infrared (1043 nm) and compared with visible light (543 nm). Scanner mirrors were used to project letters of different sizes onto the retina in both wavelengths. Subjects performed a visual test to determine the smallest letter size that was distinguishable for each wavelength for a range of defocus values. An additional optical path was used to record the retinal images of the spot after reflection in the retina and double-pass through the optical media. The best visual acuity was obtained at different defocus locations corresponding to the chromatic difference between green and infrared. Although, there was some individual variability, visual acuity was found to be similar both in visible and infrared. The use of two-photon infrared vision may have some potential applications for vision in those cases were the optical media is opaque to visible wavelengths while keeping some transparency in the infrared.

10073-27, Session 7

Towards an optical "black hole": surface reshaping for an optimal optical coupling into turbid medium

Jonathan V. Thompson, Brett H. Hokr, Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Light propagation in turbid medium is typically considered for flat or regular surfaces. However, such an approximation often does not reflect an experimental reality, and, in this report, we made the first, to our knowledge, attempt to optimize the surface of the medium to improve the optical coupling into the medium. By making conical microchannels in a turbid medium using a short-pulsed laser microdrilling, we were able to increase the photon life-time in the medium by almost 2 orders of magnitude. We extended the theory of light propagation in the turbid medium to include those geometrical effects and show its excellent agreement with the observed experimental results. We will also discuss a range of potential applications ranging from improved sensing capabilities to deep tissue optical imaging.

10073-28, Session 7

A novel algorithm for fast and efficient multifocus wavefront shaping

Parsa Omidi, Nafiseh Mohammadian, Zahra Fayyaz, Ali Hariri, Mohammadreza Nasiriavanaki, Wayne State Univ. (United States)

Wavefront shaping using spatial light modulator (SLM) is a popular method for focusing light through a turbid media, such as biological tissues. Usually, in iterative optimization methods, due to the very large number of pixels in SLM, larger pixels are formed, bins, and the phase value of the bins are changed to obtain an optimum phase map, hence a focus. In this study an efficient optimization algorithm is proposed to obtain an arbitrary map of focus utilizing all the SLM pixels or small bin sizes. The application of such methodology in dermatology, hair removal in particular, is explored and discussed.

10073-30, Session 7

Lock-in detection for linear and non-linear photoacoustic feedback focusing through scattering media

Omer Tzang, Rafael Piestun, Univ. of Colorado Boulder (United States)

Most of the feedback-based wave-front shaping techniques for focusing and imaging through scattering media require invasive feedback (inside or behind the sample) or use of angular correlations (memory effect) which exist only in limited types of samples. Several recent techniques have overcome this limitation using acoustic waves via photon tagging or via the photoacoustic (PA) effect. Our objective is to improve the performance of PA bio-imaging in terms of SNR, speed, depth penetration and resolution and use it for enhanced focusing through scattering media. We present a fully analog signal processing and lock-in detection scheme for PA as an alternative to existing digital techniques. Our motivation originates from Lock-in amplifiers' known ability to detect signals deeply buried in noise by narrowing down the detection bandwidth around a modulation frequency. In addition, the method is designed for detection of nonlinearities and to use them directly for wave-front shaping feedback. The nonlinear signals are detected by measuring high modulation harmonics in the lock-in amplifier. A pure sinusoidal excitation at the first modulation harmonic allows measurement of only the generated nonlinearities. Using a feedback based optimization with genetic algorithms, we show enhanced focusing through scattering media using linear and nonlinear PA feedbacks.

10073-31, Session 8

Exploiting multimode propagation for in-vivo endomicroscopy *(Invited Paper)*

Tomá? Cizmár, Univ. of Dundee (United Kingdom)

The turbid nature of refractive index distribution within living tissues introduces severe aberrations to light propagation thereby severely compromising image reconstruction using currently available non-invasive techniques. Numerous approaches of endoscopy, based mainly on fibre bundles or GRIN-lenses, allow imaging within extended depths of turbid tissues, however their footprint causes profound mechanical damage to all overlying regions and their imaging performance is very limited.

Progress in the domain of complex photonics enabled a new generation of minimally invasive, high-resolution endoscopes by substitution of the Fourier-based image relays with a holographic control of light propagating through apparently randomizing multimode optical waveguides. This form of endo-microscopy became recently a very attractive way to provide minimally invasive insight into hard-to-access locations within living objects.

Here, we review our fundamental and technological progression in this domain and introduce several applications of this concept in bio-medically relevant environments.

We present isotropic volumetric imaging based on advanced modes of light-sheet microscopy: by taking advantage of the cylindrical symmetry of the fibre, we facilitate the wavefront engineering methods for generation of both Bessel and structured Bessel beam plane illumination. Further, we demonstrate the first utilization of multimode fibers for imaging in living organisms. We present a new fibre-based geometry for deep tissue imaging in brain tissue of a living animal model.

Lastly, we show the development and exploitation of highly specialised fiber probes for numerous advanced bio-photonics applications including high-resolution imaging and optical manipulation.

10073-32, Session 8

Improving endoscopic imaging with disordered multi-core fiber bundles

Dan Oron, Weizmann Institute of Science (Israel); Siddharth Sivankutty, Aix-Marseille Univ. (France); Dani Kogan, Weizmann Institute of Science (Israel); Viktor Tsvirkun, Aix-Marseille Univ. (France); Esben R. Andresen, Géraud Bouwmans, Univ. des Sciences et Technologies de Lille (France); Hervé Rigneault, Aix-Marseille Univ. (France)

The periodic arrangement of core positions in multi-core fiber bundles introduces 'ghost' artifacts to endoscopic images obtained through them, whether in wide-field imaging (based on either direct imaging or speckle correlations) or in confocal scanning microscopy using wavefront shaping. Here we introduce partially disordered multi-core bundles as a means to overcome these artifacts. The benefits of their use will be discussed in the context of multiphoton scanning microscopy utilizing a spatial light modulator in the proximal end, and in the more general case of widefield imaging. We also show that both numerically and experimentally that the presence of disorder also enables to apply phase retrieval methods to characterize the phase distortion introduced due to propagation in the bundle without the need of an interferometrically stabilized reference. Thus, in addition to overcoming the challenge of ghost artifacts, disordered multi-core fibers have the potential to overcome another challenge, movement-induced phase distortions, by enabling real-time characterization of this phase distortion in reflection mode only via the proximal end.

10073-33, Session 8

Polarization control of light transmission through a multimode fiber

Wen Xiong, Yaron Bromberg, Chia Wei Hsu, Hui Cao, Yale Univ. (United States)

A multimode fiber subjected to random mode and polarization mixing represents a complex photonic system with strong coupling of spatial,

temporal, spectral and polarization degrees of freedom. By exploiting such coupling, we demonstrate a full control of the polarization state of light transmitted through a multimode fiber by adjusting the spatial profile of incident field. After applying stress to the fiber to induce strong mode and polarization mixing, we measure the polarization-dependent transmission matrices, and find the transmission eigenchannels. By launching light to specific eigenchannnels, we are able to preserve the polarization state despite strong polarization mixing in the multimode fiber, or to convert all transmitted light to the orthogonal polarization state. In addition, we show that the linearly polarized input light can be changed completely to circularly polarized output. Furthermore, arbitrary polarization states can be realized for individual spatial channels at the output by tailoring the incident wavefront of a single polarization. Such global control is possible only in the presence of strong mode mixing in the fiber. Namely, strong polarization mixing itself is not sufficient to generate arbitrary polarization states at the output. Therefore, the strong entanglement of spatial and polarization degrees of freedom is essential to achieve a complete control of polarization. Such global control of the polarization states of all output channels is more challenging than the local control of the polarization state of a single output channel.

10073-34, Session 8

Spatiotemporal control of light transmission through a multimode fiber

Wen Xiong, Yale Univ. (United States); Philipp Ambichl, Technische Univ. Wien (Austria); Yaron Bromberg, Brandon Redding, Yale Univ. (United States); Stefan Rotter, Technische Univ. Wien (Austria); Hui Cao, Yale Univ. (United States)

Optical pulses propagating through a multimode fiber with random mode mixing experience temporal broadening and distortion. Principal modes have been proposed to overcome modal dispersion. They are the eigenstates of the time delay operator and the associated eigenvalues are the delay times. Principal modes retain the spatial profiles of output fields to the first order of frequency variation. In the weak mode coupling regime, principal modes are superpositions of fiber eigenmodes with similar propagation constants. In the strong mode coupling regime, a principal mode is composed of all fiber modes with very different propagation constant, yet it has a well-defined delay time due to multipath interference, which can be controlled by adjusting the spatial profile of incident field.

The spectral bandwidth of principal modes determines the temporal width of optical pulses that can be transmitted through the multimode fiber without distortion. In the weak mode coupling regime, principal modes with short and long delay times have broader bandwidths, while in the strong mode coupling regime, the principal modes with intermediate delay times have the broadest bandwidths. The opposite behaviors reveal two distinct mechanisms that are responsible for the principal mode bandwidth in the weak and strong mode coupling regimes. We further investigate how the mode-dependent loss modifies the principal modes. Our study provides physical understanding of spatiotemporal dynamics in a multimode fiber with varying degree of mode mixing, which is important for controlling pulse propagation through a multimode fiber.

10073-35, Session 8

High-speed wavefront modulation in complex media

Sergey Turtaev, Ivo T. Leite, Tomá? Cizmár, Univ. of Dundee (United Kingdom)

Using spatial light modulators(SLM) to control light propagation through scattering media is a critical topic for various applications in biomedical imaging, optical micromanipulation, and fibre endoscopy. Having limited switching rate, typically 10-100Hz, current liquid-crystal SLM can no longer meet the growing demands of high-speed imaging. A new way based on binary-amplitude holography implemented on digital micromirror devices(DMD) has been introduced recently, allowing to reach refreshing rates of 30kHz.

Here, we summarise the advantages and limitations in speed, efficiency, scattering noise, and pixel cross-talk for each device in ballistic and diffusive regimes, paving the way for high-speed imaging through multimode fibres.

10073-36, Session 9

Nonlinear adaptive optics: Aberration correction in three photon fluorescence microscopy for mouse brain imaging. *(Invited Paper)*

David Sinefeld, Cornell Univ. (United States); Hari P. Paudel, Boston Univ. (United States); Tianyu Wang, Mengran Wang, Dimitre G. Ouzounov, Cornell Univ. (United States); Thomas G. Bifano, Boston Univ. (United States); Chris Xu, Cornell Univ. (United States)

Multiphoton fluorescence microscopy is a well-established technique for deep-tissue imaging with subcellular resolution. Three-photon microscopy (3PM) when combined with long wavelength excitation was shown to allow deeper imaging than two-photon microscopy (2PM) in biological tissues, such as mouse brain, because out-of-focus background light can be further reduced due to the higher order nonlinear excitation. As was demonstrated in 2PM systems, imaging depth and resolution can be improved by aberration correction using adaptive optics (AO) techniques which are based on shaping the scanning beam using a spatial light modulator (SLM). In this way, it is possible to compensate for tissue low order aberration and to some extent, to compensate for tissue scattering.

Here, we present a 3PM AO microscopy system for brain imaging. Soliton self-frequency shift is used to create a femtosecond source at 1675 nm and a microelectromechanical (MEMS) SLM serves as the wavefront shaping device. We perturb the 1020 segment SLM using a modified nonlinear version of three-point phase shifting interferometry. The nonlinearity of the fluorescence signal used for feedback ensures that the signal is increasing when the spot size decreases, allowing compensation of phase errors in an iterative optimization process without direct phase measurement. We compare the performance of different nonlinearities by comparing 2-, 3- and 4-photon fluorescence, resulting in exponential growth in signal improvement as the nonlinear order increases. We demonstrate the impact of the method by applying the 3PM AO system for in-vivo mouse brain imaging, showing improvement in signal at 1-mm depth inside the brain.

10073-37, Session 9

A PSF width independent of aberrations in spatially incoherent interferometry

Peng Xiao, Mathias Fink, A. Claude Boccara, Institut Langevin (France)

Optical imaging usually suffers from aberrations that are induced by various structures when imaging biological samples. Usually aberrations degrade the imaging system performances by broadening the point spread function (PSF). Unexpectedly we show that in spatially incoherent interferometry like full-filed optical coherence tomography (FFOCT), the system PSF width is almost insensitive to aberrations. Instead of considering the PSF of a classical imaging system such as a microscope, we specifically pay attention to the system PSF of interferometric imaging systems for which an undistorted wavefront from a reference beam interferes with the distorted wavefront of the object beam. By comparing the cases of scanning OCT with spatially coherent illumination, wide-field OCT with spatially coherent

illumination and FFOCT with spatially incoherent illumination, we found that in FFOCT with spatially incoherent illumination the system PSF width is almost independent of the aberrations and only its amplitude varies. This is demonstrated by theoretical analysis as well as numerical calculations for different aberrations, and confirmed by experiments with a FFOCT system. It is the first time to the best of our knowledge that such specific merit of incoherent illumination in FFOCT has been demonstrated. Based on this, the signal level is used as metric in our adaptive optics FFOCT system for retinal imaging. Only the main aberrations (defocus and astigmatism) that are dominating in eye are corrected to improve the signal to noise ratio and the high order aberrations are skipped. This would increase the correction speed thus reducing the imaging time.

10073-38, Session 9

Wavefront error detection method for Shack-Hartmann wavefront sensors

Franz Felberer, Xavier Levecq, Imagine Eyes (France); Yasmina Dahmani, Imagine Eyes (France) and Univ. Pierre et Marie Curie (France); Barbara Lamory-Bardet, Imagine Eyes (France); Pauline Treimany, Ilan Stefanon, Imagine Optic SA (France)

AO (Adaptive Optics) corrects wavefront errors to improve imaging quality in optical systems. An AO-system consist often of a SH-WFS (Shack–Hartmann wavefront sensor) and a DM (deformable mirror). The SH-WFS measures the local slopes of the wave front and iteratively calculates from these slopes the best fitting wavefront. The shape of the DM is then controlled by this information. Any error in the slope measurement (noise) will result in a residual wavefront error and hence in a reduced image quality.

The wavefront error detection method is based on the fact that the wavefront slopes have to be integrable and allows to quantify the error in the wavefront slopes measurement. The integrable wavefront derived from the measured slopes is used to re-calculate the slopes. The difference between the re-calculated slopes and the measured slopes is identified as the none-integrable noise of the slopes measurement.

The total noise is the sum of the integrable and the none-integrable noise. In order to derive a relation between the integrable and none-integrable noise 1000 measurements of the same wavefront have been taken. The average is assumed to be the noise free wave front. This wave front has been used to calculate the total noise of every single measurement.

Using this information an approximation of the total noise was found as: Total noise = None-integrable noise * 1.265.

This information can be used as an objective criterion for the quality of the wavefront measurement and to evaluate if the imagine performance is limited by the wavefront measurement or by the deformable mirror (e.g. number of actuator).

10073-39, Session 9

New sensorless wavefront sensing approach for two-photon scanning microscopy

Joel Teixeira, ONERA (France); Dorian Champelovier, Institut Fresnel (France); Jean-Marc Conan, Laurent M. Mugnier, Serge C. Meimon, ONERA (France); Serge Monneret, Hervé Rigneault, Institut Fresnel (France); Thomas Tressard, Rosa Cossart, INSERM (France); Arnaud Malvache, INSERM (France) and Institut Fresnel (France)

The landscape of biomedical research in neuroscience has changed dramatically in recent years as a result of spectacular progress in dynamic microscopy. However, the optical accessibility of deep brain structures or deeper regions of the surgically exposed hippocampus (a few 100 microns typically) remains limited, due to aberrations created by the sample. Adaptive optics can correct for these aberrations, the key issue being then the ability to perform an accurate and reliable wavefront sensing (WFS). The approach called modal sensorless WFS has the advantage of maximizing a quality metric directly on the scan images. Our developments are performed in this framework.

We first explore the limitations of standard modal sensorless WFS applied to a given transverse scan, that is with a locked focus. In a very inhomogeneous medium, such as the hippocampus, we show that this technique leads to significant WFS errors.

To overcome this issue, we then build a new approach that exploits these inhomogeneities. The new WFS strategy called Neuron Locking SensorLess (NLSL) WFS is an image quality metric optimization two-step process: 1. coarse adjustment of focus to lock on bright neurons; 2. Iterative aberration optimization including fine tuning of focus. Furthermore, we design a new metric to improve the WFS sensitivity.

The NLSL WFS approach has been implemented in our adaptive optics assisted two-photon microscope. Experiments performed on ex-vivo mouse hippocampus samples demonstrate its efficiency. In-vivo experiments are planned during summer 2016 and results will be presented at the conference.

10073-40, Session 9

Optimizing the sampling density of a wave-front sensor in adaptive optics systems - application to scanning laser ophthalmoscopy

Marie Laslandes, Matthias Salas, Christoph K. Hitzenberger, Michael Pircher, Medizinische Univ. Wien (Austria)

We present the optimization of an adaptive optics (AO) loop for retinal imaging. In most instruments, the wave-front measurement is greatly over-determined compared to the number of corrector elements. In this work we show that the number of micro-lenses of the Shack-Hartmann sensor (SH) can be significantly reduced while maintaining an efficient aberration correction. The use of fewer micro-lenses leads to higher measurement sensitivity and larger dynamic range.

Firstly, an analytical model of an AO loop is developed with matlab to characterize the link between number of actuators, number of micro-lenses and correction performance. The model takes into account possible misalignments and noise sources in the system. It allows determining the optimal characteristics of the AO elements depending on the correction needs.

Secondly, the model is validated experimentally by characterizing two different loop configurations. The correcting device (deformable mirror with 97 actuators arranged in an 11x11 grid) is alternatively driven by two SH sensors with different samplings (22x22 or 44x44 lenslet array). The obtained correction performance is equivalent for both systems. A coarser sampling requires lower light powers, leading to shorter exposure times and thus faster correction speeds.

Finally, the optimized correction loop is introduced into a scanning laser ophthalmoscope (SLO). In vivo images of foveal photoreceptors are recorded with a frame rate of 20Hz. Using standard processing of AO-SLO images including correction of in frame distortions and averaging, we demonstrate that the obtained image quality is equivalent to the current state of the art in retinal AO-imaging.

10073-41, Session 10

Adaptive optics-enabled 4pi super-resolution microscopy *(Invited Paper)*

Martin J. Booth, Univ. of Oxford (United Kingdom)

Super-resolution microscopy can be enhanced by employing a 4pi configuration, where the specimen is placed between two opposing objective lenses. Illumination and emission pass through both objectives and are combined in an interferometer. This increases the angular aperture of the system so that more fluorescence is collected and enhances the axial resolution. We have built an adaptive 4pi single molecule (STORM) microscope that incorporates a deformable mirror in each beam path to correct specimen aberrations. We discuss new control strategies for operation of such a system and discuss the implications for other super-resolution microscopes, such as STED and SIM.

10073-42, Session 10

An image-based real-time autofocus method for optical microscopy

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Defocus can severely reduce image sharpness in several optical microscopy techniques. Indeed, in wide-field methods, specimen-induced aberrations or thermal drifts of the microscope hardware can result in a walk-off between the focal plane of the detection objective and the sample. The same problem can occur in light sheet microscopy, if the illumination light sheet goes slightly off the focal plane.

For automated microscopy purposes, it is essential to measure the focal state of the system in order to provide closed-loop correction via objective motion. Two main autofocus approaches are typically used. In one method, many images are collected at different focus positions: the sharper one identifies the 'in-focus' position. The other family of techniques is based on active measurement of the distance between the objective and a reference plane (usually the coverslip), e.g. by laser triangulation. Although the latter approach can provide real-time correction, it actually provides only the position of a reference, not the one of the real sample or of the light sheet.

Here, we describe a method for one-shot measurement of defocus in an optical microscope. As this approach is image-based, it provides a direct measurement of the focal position of the plane of interest, overcoming the limitations of laser triangulation of analogous methods. We demonstrate the capabilities of our technique both in conventional wide-field microscopy and in a light sheet apparatus. This novel autofocus approach can provide a valuable tool in the field of automated microscopy.

10073-43, Session 10

Real time optimization algorithm for wavefront sensorless adaptive optics optical coherence tomography

Hans R. G. W. Verstraete, Technische Univ. Delft (Netherlands); Morgan Heisler, Myeong Jin Ju, Daniel J. Wahl, Simon Fraser Univ. (Canada); Laurens Bliek, Technische Univ. Delft (Netherlands); Jeroen Kalkman, Academisch Medisch Centrum (Netherlands); Stefano Bonora, CNR-Istituto di Fotonica e Nanotecnologie (Italy); Marinko V. Sarunic, Simon Fraser Univ. (Canada); Michel Verhaegen, Technische Univ. Delft (Netherlands); Yifan Jian, Simon Fraser Univ. (Canada)

Optical Coherence Tomography (OCT) has revolutionized modern ophthalmology, providing depth resolved images of the retinal layers in a system that is suited to a clinical environment. A limitation of the performance and utilization of the OCT systems has been the lateral resolution. Through the combination of wavefront sensorless adaptive optics with dual variable optical elements, we present a compact lens based OCT system that is capable of imaging the photoreceptor mosaic. We utilized a commercially available variable focal length lens to correct for a wide range of defocus commonly found in patient eyes, and a multi-actuator adaptive lens after linearization of the hysteresis in the piezoelectric actuators for aberration correction to obtain near diffraction limited imaging at the retina. A parallel processing computational platform permitted real-time image acquisition and display. The Data-based Online Nonlinear Extremum seeker (DONE) algorithm was used for real time optimization of the wavefront sensorless adaptive optics OCT, and the performance was compared with a coordinate search algorithm. Cross sectional images of the retinal layers and en face images of the cone photoreceptor mosaic acquired in vivo from research volunteers before and after WSAO optimization are presented. Applying the DONE algorithm in vivo for wavefront sensorless AO-OCT demonstrates that the DONE algorithm succeeds in drastically improving the signal while achieving a computational time of 1 ms per iteration, making it applicable for high speed real time applications.

10073-44, Session 10

Pupil segmentation adaptive optics for in-vivo mouse retinal fluorescence imaging

Christine Huang, Daniel J. Wahl, Simon Fraser Univ. (Canada); Stefano Bonora, CNR-Istituto di Fotonica e Nanotecnologie (Italy); Yifan Jian, Marinko V. Sarunic, Simon Fraser Univ. (Canada)

Characterization of diseases and development of novel therapies for vision impairment is facilitated through small animal research. Aberrations introduced by the high numerical aperture of the mouse eye make adaptive optics (AO) vital for cellular resolution in longitudinal studies. We have implemented wavefront-sensorless AO using a pupil segmentation approach to correct aberrations in the mouse eye. Our lens-based AO system uses a segmented MEMS deformable mirror (DM) that is well-adapted for pupil segmentation. We demonstrate the system using a fluorescent bead phantom and compare the results to other sensorless AO approaches. The results with pupil segmentation are competitive with our previous results using a modal hill-climbing algorithm, and demonstrate potential for in-vivo fluorescence mouse retinal imaging. Pupil segmentation was performed by acquiring an image of the sample for each mirror segment while deflecting all other segments. The relative shift of the image acquired with a particular mirror segment with respect to the reference (central segment) revealed the wavefront slope in the corresponding region of the pupil. The image shift from each mirror segment was used to calculate the amplitude of the Zernike coefficients that were applied to the DM to correct the wavefront across the full pupil. Even small motions from a living mouse during the overall acquisition can corrupt the wavefront reconstruction. The motion artifact was reduced by rapidly acquiring a reference image along with each pupil segment image. To further decrease the effect of motion artifacts, we alternated between the reference and each mirror segment within a frame.

10073-45, Session 10

Probing neural circuits with shaped light *(Invited Paper)*

Na Ji, Howard Hughes Medical Institute (United States)

To understand computation in the brain, one needs to understand the input-output relationships for neural circuits and the anatomical and functional relationships between individual neurons therein. Optical microscopy has emerged as an ideal tool in this quest, as it is capable of recording the activity of neurons distributed over millimeter dimensions with sub-micron spatial resolution. I will describe how we use concepts in astronomy and optics to develop next-generation microscopy methods for imaging neural circuits at higher resolution, greater depth, and faster speed. By shaping the wavefront of the light, we have achieved synapse-level spatial resolution through the entire depth of primary visual cortex, optimized microendoscopes for imaging deeply buried nuclei, and developed a video-rate (30 Hz) volumetric imaging method. We apply these methods to understanding neural circuits, using the mouse primary visual cortex as our model system.

10073-46, Session 11

Advanced retinal imaging *(Invited Paper)*

Jennifer Hunter, Univ. of Rochester (United States)

Microscopic imaging of the living retina enables non-invasive exploration of cellular structure and processes in the normal and diseased eye. By employing adaptive optics to correct the eye's optical imperfections (Liang et. al, 1997) and using light reflected from various retinal layers, we can visualize the photoreceptor mosaic, retinal vasculature and the retinal nerve fiber layer in the living eye (Roorda and Duncan, 2015). Single-photon fluorescence techniques have allowed us to see the retinal pigment epithelium (RPE) cell mosaic (Morgan, Dubra et. al, 2009) and, with the use of extrinsic fluorophores, ganglion cells (Geng et. al, 2011) and other retinal neurons. Time-resolved imaging of genetically-encoded calcium indicators expressed in ganglion cells can provide useful information about their response to varying visual stimuli and their role in vision (Yin et. al, 2014). Two-photon excited fluorescence imaging of intrinsic fluorescence has made it possible to non-invasively image the ganglion cell mosaic and other inner retinal cells which have evolved to be transparent in visible light (Sharma, Williams et. al, 2016). Non-linear imaging techniques can also interrogate molecular processes that are inaccessible with conventional reflectance and fluorescence ophthalmoscopy such as the visual cycle within rods and cones (Sharma, Schwarz et. al, 2016). All of these methods are critical for understanding retinal structure and function in health and disease, in evaluating new treatments as well as accelerating the development of novel methods for vision restoration.

10073-47, Session 11

Modelling of optical aberrations caused by light propagation in mouse cranial bone using second harmonic generation imaging

Kayvan F. Tehrani, Peter Kner, Luke J. Mortensen, The Univ. of Georgia (United States)

Multi-photon imaging through the bone to image into the bone marrow or even the brain is an emerging need in the scientific community. Due to the highly scattering nature of bone, mechanical bone thinning or removal is typically required to enhance the resolution and signal intensity of the imaging plane. This is due to optical aberrations and scattering that significantly affect the resolution and signal to noise ratio of deep tissue microscopy. Multi-photon microscopy uses long wavelength (near-infrared and infrared) excitation light to reduce the effects of scattering. However it is still susceptible to optical aberrations and scattering since the light propagates through several layers of media with inhomogeneous indices of refraction. Mechanical removal of bone is highly invasive, laborious, and cannot be applied in experiments where imaging inside of the bone is desired. Adaptive optics technology can compensate for these optical aberrations and potentially restore the diffraction limited point spread function of the system even in deep tissue. To design an adaptive optics system, a priori knowledge of the sample structure assists selection of the proper correction element and sensing methods. In this work we present the characterization of optical aberrations caused by mouse cranial bone, using second harmonic generation imaging of bone collagen. We simulate light propagation through the bone and calculate aberrations that can be corrected using a deformable mirror. We calculate the residual wavefront error and estimate the number of segments a correcting element requires to effectively correct aberrations for imaging through bone.

10073-48, Session 11

Wavefront sensorless adaptive optics versus sensor-based adaptive optics for in vivo fluorescence retinal imaging

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Adaptive optics (AO) is essential for achieving diffraction limited resolution in large numerical aperture (NA) in-vivo retinal imaging in small animals. Cellular-resolution in-vivo imaging of fluorescently labeled cells is highly desirable for studying pathophysiology in animal models of retina diseases in pre-clinical vision research. Currently, wavefront sensor-based (WFS-based) AO is widely used for retinal imaging and has demonstrated great success. However, the performance can be limited by several factors including common path errors, wavefront reconstruction errors and an ill-defined reference plane on the retina. Wavefront sensorless (WFS-less) AO has the advantage of avoiding these issues at the cost of algorithmic execution time. We have investigated WFS-less AO on a fluorescence scanning laser ophthalmoscopy (fSLO) system that was originally designed for WFS-based AO. The WFS-based AO uses a Shack-Hartmann WFS and a continuous surface deformable mirror in a closed-loop control system to measure and correct for aberrations induced by the mouse eye. The WFS-less AO performs an open-loop modal optimization with an image quality metric. After WFS-less AO aberration correction, the WFS was used as a control of the closed-loop WFS-less AO operation. We can easily switch between WFS-based and WFS-less control of the deformable mirror multiple times within an imaging session for the same mouse. This allows for a direct comparison between these two types of AO correction for fSLO. Our results demonstrate volumetric AO-fSLO imaging of mouse retinal cells labeled with GFP. Most significantly, we have analyzed and compared the aberration correction results for WFS-based and WFS-less AO imaging.

10073-49, Session 11

Remote axial displacement of spatiotemporal focused patterns through neural systems *(Invited Paper)*

Valentina Emiliani, Univ. Paris Descartes (France)

Two-photon (2P) excitation can be combined with phase-modulation approaches, such as computer-generated holography (CGH), to efficiently distribute light into two-dimensional, axially confined, user-defined shapes. Applications include lithography, uncaging, optogenetics and fast functional imaging. However, a linear proportionality between lateral shape area and axial extent degrades axial precision for cases demanding extended

lateral patterning.To address this limitation, we previously combined CGH with temporal focusing (TF) to stretch laser pulses outside of the focal plane, which combined with 2P's nonlinear fluorescence dependence, axially confines fluorescence regardless of lateral extent. However, this configuration restricts nonlinear excitation to a single spatiotemporal focal plane, which is the objective focal plane.

Here we report a novel optical scheme enabling remote axial displacement and simultaneous generation of spatiotemporally focused pattern at multiple planes using two spatial light modulators to independently control transverse- and axial-target light distribution. This approach enabled simultaneous axial translation of single or multiple spatiotemporal focused patterns across the sample volume, while achieving the axial confinement of temporal focusing. We utilized the system's novel capability to dissect the functional connectivity between axially distinct neuronal layers in the mice retina.

Finally, we demonstrated that TF enables robust light propagation trough optically and physiologically diverse neural systems including mice brain, zebrafish larva brain and mice retina.

10073-50, Session PMon

Novel single-fibre probes for advanced biophotonic applications

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Optical imaging within turbid media such as biological tissue is typically limited to penetration depths of up to hundreds of micrometres due to multiple scattering events occurring at inhomogeneities in the volume of the sample, which limit the attainable imaging resolution. Applications demanding imaging with optical resolution deep within the turbid sample thus require the use of an endoscopic probe, which naturally can pose constraints by damaging the surrounding environment.

Recent advances in holographic beam-shaping in multimode fibres have shown a great potential for devising minimally invasive probes comprised of a single optical fibre. Until now, most work in this field has been grounded on the well-established technology of optical fibre manufacturing, which was developed mainly for applications in optical communications. However, qualities such as low-cost and low-attenuation which are greatly appreciated for kilometre-scale networks are in general not beneficial for imaging applications - where the scales involved are in the order of centimetres - as they typically come with a cost in unnecessarily large cladding dimensions and low NA.

In this contribution, we will introduce a new concept of optical fibres with optimised design for applications as single-fibre imaging probes, and high-performance imaging and optical manipulation which will pave the way for the further development of advanced biophotonics applications.

10073-51, Session PMon

Time-reversal of a pulsed laser with wavefront shaping

YoonSeok Baek, KyeoReh Lee, YongKeun Park, KAIST (Korea, Republic of)

We demonstrate a time reversal of a pulsed light using wavefront shaping. Spatio-temporal profiles of light are phase conjugated using a single mode reflector. The lights are modulated to be focused in space and in time, whose reverse propagation generates a phase conjugated signal. We demonstrate phase conjugations of pulsed lights of various sptaio-temporal signals through scattering layer.

10073-52, Session PMon

Rapid lattice light sheet Raman microscopy

Seongjun Park, Institute for Basic Science (Korea, Republic of); Sung Ho Lee, Seonik Lee, Bo-Kung Kim, Sung Chul Bae, Ulsan National Institute of Science and Technology (Korea, Republic of)

Raman microscopy is a valuable tool for visualizing the distribution of molecular species in cells without any labeling. Due to the low Raman scattering, Raman microscopy at high temporal resolution has been challenging for studying most dynamic processes of living samples. Here, we present a rapid lattice light sheet Raman microscopy, which uses an ultrathin lattice light sheets perpendicular to the detection axis to illuminate the sample and a narrow band filter to observe the scattering intensity at a particular Raman peak of interest. The feasibility was confirmed with a composite of polystyrene beads and lipid droplets in agar.

10073-53, Session PMon

Laser beam focusing through the scattering medium by means of adaptive optics

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Laser beam propagation through the scattering suspension of polystyrene microspheres in distilled water was studied both numerically and experimentally. The distorted beam was analyzed by Shack-Hartmann sensor. The measured local slopes of the Poynting vector was compensated for by means of bimorph deformable mirror with 48 electrodes in order to improve laser beam focusing. Numerical and experimental investigation of the focusing improvement of the laser beam propagated through the scattering medium was performed.

10074-1, Session 1

Optofluidic time-stretch quantitative phase microscopy for high-throughput label-free single-cell screening *(Invited Paper)*

Baoshan Guo, Cheng Lei, The Univ. of Tokyo (Japan); Takuro Ito, Japan Science and Technology Agency (Japan); Yiyue Jiang, Yasuyuki Ozeki, Keisuke Goda, The Univ. of Tokyo (Japan)

The ability to sift through a large heterogeneous population of cells is of paramount importance in a diverse range of biomedical and green applications. Furthermore, the capability of identifying various features of cells in a label-free manner is useful for high-throughput screening. Here we present optofluidic time-stretch quantitative phase microscopy for high-throughput label-free single-cell screening. This method is based on an integration of a hydrodynamic-focusing microfluidic chip, an optical time-stretch microscope for high-speed imaging with a spatial resolution of ~800 nm at a frame rate of ~10 million frames per second, and a digital image processor for image-based characterization, classification, and statistical analysis of biological cells such as blood cells and microalgae. It provides both the opacity (amplitude) and thickness (phase) content of every cell at a high throughput of ~10,000 cells per second. This method is expected to be effective for a diverse range of applications such as cancer detection and biofuel production.

10074-2, Session 1

Label-free tomographic reconstruction of optically thick structures using GLIM

Mikhail E. Kandel, Ghazal N. Kouzehgarani, Tan H. Ngyuen, Martha U. Gillette, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Although the contrast generated in transmitted light microscopy is due to the elastic scattering of light, multiple scattering scrambles the image and reduces overall visibility. To image both thin and thick samples, we turn to gradient light interference microscopy (GLIM) to simultaneously measure morphological parameters such as cell mass, volume, and surfaces as they change through time. Because GLIM combines multiple intensity images corresponding to controlled phase offsets between laterally sheared beams, incoherent contributions from multiple scattering are implicitly cancelled during the phase reconstruction procedure. As the interfering beams traverse near identical paths, they remain comparable in power and interfere with optimal contrast. This key property lets us obtain tomographic parameters from wide field z-scans after simple numerical processing. Here we show our results on reconstructing tomograms of bovine embryos, characterizing the time-lapse growth of HeLa cells in 3D, and preliminary results on imaging much larger specimen such as brain slices.

10074-3, Session 1

Halo-free phase contrast imaging

Tan H. Nguyen, Mikhail E. Kandel, Haadi M. Shakir, Catherine Best, Minh N. Do, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

The phase contrast (PC) method is one of the most impactful developments in the four-century long history of microscopy. It allows for intrinsic, nondestructive contrast of transparent specimens, such as live cells. However, PC is plagued by the halo artifact, a result of insufficient spatial coherence in the illumination field, which limits its applicability. We present a new approach for retrieving halo-free phase contrast microscopy (hfPC) images by upgrading the conventional PC microscope with an external interferometric module, which generates sufficient data for reversing the halo artifact. Measuring four independent intensity images, our approach first measures haloed phase maps of the sample. We solve for the halo-free sample transmission function by using a physical model of the image formation under partial spatial coherence. Using this halo-free sample transmission, we can numerically generate artifact-free PC images. Furthermore, this transmission can be further used to obtain quantitative information about the sample, e.g., the thickness with known refractive indices, dry mass of live cells during their cycles. We tested our hfPC method on various control samples, e.g., beads, pillars and validated its potential for biological investigation by imaging live HeLa cells, red blood cells, and neurons.

10074-4, Session 1

Holographic trapping of non-spherical particles with 3D refractive index measurements

Kyoohyun Kim, YongKeun Park, KAIST (Korea, Republic of)

Holographic optical tweezers (HOTs) have been utilized for trapping microscopic particles in three dimensions with multiple foci generated by wavefront shaping of light, which can manipulate three-dimensional (3-D) positions of colloidal particles as well as exerting an optical force on particles. So far, most experiments using HOTs have been conducted for trapping spherical particles because optical principles can easily predict optical forces and the responding motion of microspheres. For non-spherical particles, however, calculation of optical forces and torques exerting on samples is very complicated, and the orientation control of non-spherical particles is limited since the non-spherical particles tend to align along the optic axis of the trapping beam.

Here, we propose and experimentally demonstrate 3-D trapping of non-spherical particles by wavefront shaping of light based on the measurement of 3-D refractive index (RI) distribution of samples. The 3-D RI distribution of non-spherical particles was measured by optical diffraction tomography and the phase hologram which can generate stable optical traps for the samples was calculated by iterative 3-D Gerchberg-Saxton algorithm from the measured 3-D RI distribution. We first validate the proposed method for stable trapping and orientation control of 2-?m colloidal PMMA ellipsoids. The proposed method is also exploited for rotating, folding and assembly of red blood cells.

10074-5, Session 1

Phase retrieval and 3D imaging in gold nanoparticles based fluorescence microscopy *(Invited Paper)*

Tali Ilovitsh, Asaf Ilovitsh, Aryeh M. Weiss, Rinat Meir, Zeev Zalevsky, Bar-Ilan Univ. (Israel)

Optical sectioning microscopy can provide highly detailed three dimensional (3D) images of biological samples. However, it requires acquisition of many images per volume, and is therefore time consuming, and may not be suitable for live cell 3D imaging. We propose the use of the modified Gerchberg-Saxton phase retrieval algorithm to enable full 3D imaging of gold nanoparticles tagged sample using only two images. The reconstructed field is free space propagated to all other focus planes using post processing, and the 2D z-stack is merged to create a 3D image of the sample with high fidelity. Because we propose to apply the phase retrieving on nano particles, the regular ambiguities typical to the Gerchberg-Saxton algorithm, are eliminated. The proposed concept is then further enhanced

also for tracking of single fluorescent particles within a three dimensional (3D) cellular environment based on image processing algorithms that can significantly increases localization accuracy of the 3D point spread function in respect to regular Gaussian fitting. All proposed concepts are validated both on simulated data as well as experimentally.

10074-6, Session 1

White-light quantitative phase imaging unit

YoonSeok Baek, KyeoReh Lee, Jonghee Yoon, Yongkeun Park, KAIST (Korea, Republic of)

We present the white-light quantitative phase imaging unit (WQPIU). The WQPIU enables quantitative phase imaging (QPI) under white light illumination. With its compact interferometric design, the WQPIU can be utilized on microscope platforms. The principle of the WQPIU is based on lateral shearing interferometry and phase shifting interferometry. Its performance has been demonstrated with polystyrene beads, human red blood cells, HeLa cells and mouse white blood cells. We expect the WQPIU to broaden the scope of QPI in biological sciences as a powerful and simple imaging tool.

10074-7, Session 1

Single-exposure quantitative phase imaging in color-coded LED microscopy

Wonchan Lee, Daeseong Jung, Chulmin Joo, Yonsei Univ. (Korea, Republic of)

Quantitative phase-gradient or phase imaging in LED microscopy has been recently demonstrated. The methods enable measurement of phase distribution of transparent specimens in a simple and cost-effective manner, but require multiple image acquisitions with different source or pupil configurations to improve phase accuracy.

Here, we demonstrate a strategy for single-shot quantitative phase imaging in color-coded LED microscopy. We employ a circular LED illumination pattern that is trisected into subregions with equal area, assigned to red, green and blue colors, respectively. Additional color filter is also employed to mitigate the color leakage of light into different color channels of the image sensor. Image acquisition with a color image sensor and subsequent computation based on the weak object transfer function allow for quantitative amplitude and phase measurements of a specimen. We describe computational model and single-shot quantitative phase imaging capability of our method by presenting phase images of calibrated phase sample and dynamics of cells. Phase measurement accuracy is validated with pre-characterized phase plate, and single-shot phase imaging capability is demonstrated with time-lapse imaging of cells acquired at 30 Hz.

10074-8, Session 1

Sparsity assisted approach for imaging from laser speckle

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Optical imaging through scattering medium has remarkable interests due to wide range of applications in the areas like diagnosis of the scatterer, imaging through turbid medium, deep tissue microscopy, biomedical imaging etc. The presence of a highly scattering medium significantly scrambles the phase and amplitude of light coming from the object into random speckle pattern, where the object recovery is considered to be difficult or impossible. A significant number of techniques were developed in recent years to address this fundamental and practical imaging obstacle based on time gating, inverse scattering, wave front shaping, ghost imaging, speckle correlation etc. Recent developments in correlation based techniques and iterative techniques provide new opportunities to deal with this imaging scenario. Very recently we have developed non-invasive imaging technique through scattering medium by utilizing the correlation approach in conjunction with interferometry.

Existing correlation based approaches uses interferometric method for the retrieval of the two point complex correlation function by making use of field or intensity based interferometers. In this paper, we propose a non-interferometric technique for imaging through scattering layer using speckle autocorrelation assisted with sparsity enhanced iterative phase reconstruction. The use of sparsity assisted approach in combination with speckle correlation in imaging gives the potential to retrieve the complex correlation function from random speckle pattern using direct intensity measurements. The advantage of the technique is the retrieval of the complex correlation function from rather simple intensity measurements and thereby provides the potential to recover the complex valued object in its entirety (i.e. both amplitude and phase). Proof of principle experiment is carried out for the proposed technique and imaging of an object from the laser speckle is demonstrated.

10074-9, Session 2

Super-resolution fluorescence imaging using quantitative phase imaging *(Invited Paper)*

Pierre Bon, Institut d'Optique Graduate School (France) and Univ. Bordeaux (France); Jeanne Linares, Bordeaux Univ. (France); Laurent Cognet, Ctr. National de la Recherche Scientifique (France)

Quantitative Phase Imaging has been developed for label-free purpose. However, the use of QPI in the scope of fluorescence microscopy may give very interesting results. I will show in my presentation that this concept is very useful in the world of super-resolution for both drift compensation and 3D super-resolution. Indeed, a simultaneous measurement of both intensity and phase gives us the opportunity the localize in 3D sub-diffraction particles at very high frame-rate and potentially without photon loss.

10074-10, Session 2

Multi-wavelength digital holographic microscopy based on spatial light modulation

Gaël Nardin, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Tristan Colomb, Yves Emery, Lyncée Tec SA (Switzerland); Christophe Moser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

We demonstrate the potential of Spatial Light Modulation (SLM) for the spectral control of a broadband light source in Digital Holographic Microscopy (DHM). DHM is an off-axis interferometric measurement technique where the spectral properties of the light source are of upmost importance. The use of a SLM in a "pulse-shaper" configuration enables fast, mechanical motion-free control over the bandwidth and wavelength of the source. As an application example, we generate sequences of different wavelengths for a hierarchical Optical Phase Unwrapping (OPU) algorithm. OPU algorithms are useful to solve the phase-ambiguity problem in the case of rough or high-aspect-ratio structures, where numerical phase unwrapping generally fails. The versatile setup enables the generation of a sequence of wavelengths adapted to the structure height and noise conditions, to extend

the phase-unambiguous range to the coherence length of the spectrally controlled source. We show the unambiguous measurement of structures up to 50 microns tall, while maintaining the interferometric resolution of DHM.

10074-11, Session 2

High-speed and high-resolution quantitative phase imaging with digital-micromirror device-based illumination

Renjie Zhou, Di Jin, Zahid Yaqoob, Peter T. C. So, Massachusetts Institute of Technology (United States)

Due to the large number of available mirrors, the patterning speed, low-cost, and compactness, digital-micromirror devices (DMDs) have been extensively used in biomedical imaging system. Recently, DMDs have been brought to the quantitative phase microscopy (QPM) field to achieve synthetic-aperture imaging and tomographic imaging. Last year, our group demonstrated using DMD for QPM, where the phase-retrieval is based on a recently developed Fourier ptychography algorithm. In our previous system, the illumination angle was varied through coding the aperture plane of the illumination system, which has a low efficiency on utilizing the laser power. In our new DMD-based QPM system, we use the Lee-holograms, which is conjugated to the sample plane, to change the illumination angles for much higher power efficiency. Multiple-angle illumination can also be achieved with this method. With this versatile system, we can achieve FPM-based high-resolution phase imaging with 250 nm lateral resolution using the Rayleigh criteria. Due to the use of a powerful laser, the imaging speed would only be limited by the camera acquisition speed. With a fast camera, we expect to achieve close to 100 fps phase imaging speed that has not been achieved in current FPM imaging systems. By adding reference beam, we also expect to achieve synthetic-aperture imaging while directly measuring the phase of the sample fields. This would reduce the phase-retrieval processing time to allow for real-time imaging applications in the future.

10074-12, Session 2

A direct holographic method exploiting speckle-correlation scattering matrix

KyeoReh Lee, YongKeun Park, KAIST (Korea, Republic of)

The ‘holography’ measures the both amplitude and wavefront of incident light, while ‘photography’ cannot obtain the wavefront information, which gives volumetric and depth perception. However, direct holography has not been achieved in optical regime because of the insufficient bandwidth of current electronics unfortunately. Though reference-field-assisted interferometric methods have been popularly used in wide disciplines, introducing a reference field raises several fundamental and practical issues. To remedy the issues, several reference-free holographic methods have been proposed including Shack-Hartmann type sensor, transport-of-intensity equation, ptycography, and Fienup-type iterative algorithms, most of them have to sacrifice the generality of incident field by introducing assumptions, and/or require multiple measurements.

Here, we propose an optical diffuser as a holographic lens exploiting the speckle-correlation scattering matrix with the spatially chaotic property of diffused light. By passing through a calibrated turbid layer, the field information can be directly retrieved from the single speckle pattern snapshot. Using the proposed concept, we experimentally demonstrate a holographic camera by simply placing a commercial diffuser in front of camera successfully. We also perform numerical refocusing based upon a single captured hologram of real target. Our holographic method is simple and compact; and does not require any assumptions, additional reference light, nor multiple measurements, which is the closest realization of the ideal holographic camera. We expect this method can also be translated to other frequency regime such as infrared, X-ray, and electron beam.

10074-13, Session 2

A compact, robust, high throughput digital holographic microscope with high sensitivity

Jay L. Nadeau, Manuel Bedrossian, California Institute of Technology (United States); Christian A. Lindensmith, Jet Propulsion Lab. (United States)

Digital holographic microscopy (DHM) is ideal for detection of bacteria in complex fluids where low-level detection is needed: examples are blood, cerebrospinal fluid, food products, or milk. DHM allows the quantitative imaging of live samples in their native environments, without the need for concentration or staining. Off-axis DHM offers a six dimensional imaging technique of large sample volumes due its 3D imaging capabilities (physical dimensions) in real time (temporal dimension), as well as its ability to measure both the intensity of light absorbed and OPD (index of refraction times thickness). In this work, we present the design of a compact and robust off-axis DHM and results from imaging samples spiked with 0-108 bacteria/mL. Guidelines for imaging times required to achieve different levels of detection sensitivity are presented, and an upper limit to bacterial counting is also shown, illustrating the sample turbidity at which hologram reconstruction is no longer possible. The instrument is an ‘all-in-one’ fully autonomous off-axis DHM with its own electronics and sample loading and unloading abilities. The instrument contains the optical components in a common path in order to minimize the effects of misalignment, while offering the benefits of off-axis holography. The sensitivity of the off-axis DHM instrument was quantified both theoretically and experimentally and found to be more than an order of magnitude more sensitive than other conventional instruments with similar optical specifications. The high sensitivity, compactness, and robustness of this off-axis DHM have major implications for field diagnostics and high-throughput bacterial screening of liquid samples.

10074-14, Session 2

Adaptive flow-field measurements using digital holography

Jürgen W. Czarske, Nektarios Koukourakis, Bob Fregin, Jörg König, Lars Büttner, TU Dresden (Germany)

Wavefront shaping and digital holography have become suitable tools for computational imaging. They enable to correct the influence of fluctuating phase boundaries or thin phase disturbances on flow-field measurements. In this paper, we present flow velocity measurements using particle image velocimetry (PIV). PIV is based on the correlation of images of tracer particles and requires accurate determination of the particle positions. Optical distortions and aberrations, e.g. from temporally or spatially varying phase boundaries, can deteriorate the measurements significantly or may even prevent the measurement to be performed successfully at all. Quantitative phase imaging in combination with wavefront correction using spatial light modulators enables the improvement of the quality of the particle image. The reference signal for the correction procedure can be gained by realizing a guide star. In this paper, we introduce a distributed Fresnel guide-star technique, which requires just one optical access and is completely non-invasive. Flow measurements were performed in a microchannel with 500 μm side length. Introducing an exemplary defocus aberration leads to a shift of the measurement volume and hence to a velocity measurement uncertainty of up to 15 %, if no correction is used. With correction, the uncertainty is reduced to about 1 %. Accurate velocity measurements also have been carried out through scattering media, such as a rough foil generating a speckle pattern. Perspectives lie in flow measurements in two-phase flows or in biological tissue.

10074-15, Session 2

Versatile quantitative phase imaging system applied to high-speed, low noise and multimodal imaging

Antoine Federici, Sherazade Aknoun, PHASICS S.A. (France); Julien Savatier, Institut Fresnel (France); Benoit F. Wattellier, PHASICS S.A. (France)

Quadriwave lateral shearing interferometry (QWLSI) is a well-established quantitative phase imaging (QPI) technique based on the analysis of interference patterns of four diffraction orders by an optical grating set in front of an array detector [1]. As a QPI modality, this is a non-invasive imaging technique which allow to measure the optical path difference (OPD) of semi-transparent samples.

We present a system enabling QWLSI with high-performance sCMOS cameras [2] and apply it to perform high-speed imaging, low noise as well as multimodal imaging. This modified QWLSI system contains a versatile optomechanical device which images the optical grating near the detector plane. Such a device is coupled with any kind of camera by varying its magnification.

In this paper, we study the use of a sCMOS Zyla5.5 camera from Andor along with our modified QWLSI system.

We will present high-speed live cell imaging, up to 200Hz frame rate, in order to follow intracellular fast motions while measuring the quantitative phase information.

The structural and density information extracted from the OPD signal is complementary to the specific and localized fluorescence signal [2]. In addition, QPI detects cells even when the fluorophore is not expressed. This is very useful to follow a protein expression with time.

The 10 μm spatial pixel resolution of our modified QWLSI associated to the high sensitivity of the Zyla5.5 enabling to perform high quality fluorescence imaging, we have carried out multimodal imaging revealing fine structures cells, like actin filaments, merged with the morphological information of the phase.

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10074-16, Session 2

Interference fringe-based ptychography

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Ptychography is an approach to coherent diffractive imaging in which the amplitude and phase of an unknown object are retrieved by recording a series of diffraction patterns of an object using a set of spatially constrained incident fields. The recorded diffraction patterns are fed into an iterative algorithm to reconstruct amplitude and phase of both the object exit surface wave and the probe field. The most often used approach to ptychography employs a localized illumination spot, which should have a constant field profile in all the diffraction recordings.

We present a novel probe design for near-field structured illumination ptychography, where interference fringes that result from overlapping two coherent beams are used to probe the sample. Instead of scanning the entire probe over the sample, only the phase of the fringes is shifted. An advantage of this approach is the simplicity and robustness of scanning the phase of an interference pattern compared to sample movement. The electric field of the probe can be reconstructed directly from the measured diffraction recordings, which strongly relaxes stability requirements on the setup. Amplitude and phase of the object can be retrieved using a standard extended ptychographical iterative engine in the Fresnel domain.

We demonstrate the concept of interference ptychography with a free-space optical setup on a USAF target, demonstrating a resolution of less than 2.2 microns. We also developed a compact fully-fiber-integrated imaging system with which we image prepared biological samples. Only 8 images are required for reconstruction, enabling a high reconstruction speed.

10074-17, Session 2

Confocal reflectance quantitative phase microscope system for cellular membranes dynamics study

Vijay Raj Singh, Zahid Yaqoob, Peter T. C. So, Massachusetts Institute of Technology (United States)

Quantitative phase microscopy (QPM) techniques developed so far primarily belongs to high speed transmitted light based systems that has enough sensitivity to resolve membrane fluctuations and dynamics, but has no depth resolution. Therefore, most biomechanics studies using QPM today is confined to simple cells, such as RBCs, without internal organelles. An important instrument that will greatly extend the biomedical applications of QPM is to develop next generation microscope with 3D capability and sufficient temporal resolution to study biomechanics of complex eukaryotic cells including the mechanics of their internal compartments. For eukaryotic cells, the depth sectioning capability is critical and should be sufficient to distinguish nucleic membrane fluctuations from plasma membrane fluctuations. Further, this microscope must provide high temporal resolution since typical eukaryotes membranes are substantially stiffer than RBCs. A confocal reflectance quantitative phase microscope is presented based on multi-pinhole scanning, with the capabilities of higher temporal resolution and sensitivity for nucleic and plasma membranes of eukaryotic cells. System hardware is developed based on an array of confocal pinhole generated by using the 'ON' state of subset of micro-mirrors of digital micro-mirror device (DMD, from Texas Instruments) and high-speed raster scanning provides 14ms imaging speed in wide-field mode. A common path interferometer is integrated at the imaging arm for detection of specimens' quantitative phase information. Theoretical investigation of quantitative phase reconstructed from system is investigated and application of system is presented for dimensional fluctuations measurements of both cellular plasma and nucleic membranes of embryonic stem cells.

10074-50, Session 2

A model for quantifying contrast enhancement in optical coherence tomography (OCT)

Yonatan Winetraub, Elliott D. SoRelle, Orly Liba, Adam de la Zerda, Stanford Univ. (United States)

We have developed a model to accurately quantify the signals produced by exogenous scattering agents used for contrast-enhanced Optical Coherence Tomography (OCT). This model predicts distinct concentration-dependent signal trends that arise from the underlying physics of coherence-based detection. Accordingly, we show that real scattering particles can be described as simplified ideal scatterers with modified scattering intensity and concentration. The relation between OCT signal and particle

concentration is approximately linear at concentrations lower than 0.8 particles per imaging voxel. However, at higher concentrations, interference effects cause signal to increase with a square root dependence on the number of particles within a voxel. Finally, high particle concentrations cause enough light attenuation to saturate the detected signal. Predictions were validated by comparison with measured OCT signals from gold nanorods (GNRs) prepared in water at concentrations ranging over five orders of magnitude (50 fM to 5nM). In addition, we validated that our model accurately predicts the signal responses of GNRs in highly heterogeneous scattering environments including whole blood and living animals. By enabling particle quantification, this work provides a valuable tool for current and future contrast-enhanced in vivo OCT studies. More generally, the model described herein may be applied for detected signals in other modalities that rely on coherence-based detection or are susceptible to interference effects, most notably medical ultrasound. Thus, our model may enable quantitative interpretation of ultrasound contrast agents including gas-filled microbubbles.

10074-19, Session 3

Measuring dynamic membrane fluctuations in cell membrane using quantitative phase imaging

SangYun Lee, Kyoohyun Kim, YongKeun Park, KAIST (Korea, Republic of)

There is a strong correlation between the dynamic membrane fluctuations and the biomechanical properties of living cells. The dynamic membrane fluctuation consists of submicron displacements, and can be altered by changing the cells' pathophysiological conditions.

These results have significant relevance to the understanding of RBC biophysics and pathology, as follows. RBCs must withstand large mechanical deformations during repeated passages through the microvasculature and the fenestrated walls of the splenic sinusoids. This essential ability is diminished with senescence, resulting in physiological destruction of the aging RBCs. Pathological destruction of the red cells, however, occurs in cells affected by a host of diseases such as spherocytosis, malaria, and Sickle cell disease, as RBCs depart from their normal discoid shape and lose their deformability. Therefore, quantifying the RBC deformability insight into a variety of problems regarding the interplay of cell structure, dynamics, and function. Furthermore, the ability to monitor mechanical properties of RBCs is of vital interest in monitoring disease progression or response to treatment as molecular and pharmaceutical approaches for treatment of chronic diseases.

Here, we present the measurements of dynamic membrane fluctuations in live cells using quantitative phase imaging techniques. Measuring both the 3-D refractive index maps and the dynamic phase images of live cells are simultaneously measured, from which dynamic membrane fluctuation and deformability of cells are precisely calculated. We also present its applications to various diseases ranging from sickle cell diseases, babesiosis, and to diabetes.

10074-20, Session 3

Hilbert phase dynamometry for real-time imaging of cell traction

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Understanding the forces that cells exert on their surroundings is of central importance for elucidating fundamental biological phenomena including cell motility, proliferation, and differentiation. However, measuring these cell-generated forces on the extracellular matrix remains a challenge. We present a new approach to these measurements, Hilbert phase dynamometry, that promises continuous, nondestructive force field imaging over a broad range of space and time scales that may be performed in parallel with other optical measurements. Using this technique, we present data on cell growth and the force generation by mesenchymal stem cells in the process of differentiating into osteocytes and adipocytes.

10074-18, Session 4

Phase unwrapping and two wavelength multiplexing for quantitative phase interferometry using a portable module

Nir A. Turko, Natan T. Shaked, Tel Aviv Univ. (Israel)

The vast majority of quantitative phase imaging techniques face the same major issue of phase unambiguity. The phase of light is 2ϖ-periodic, and as such can only be extracted in a small range of typically 0.4 μm to 0.7μm in the visible range. As most of the samples in biology and metrology are much higher, phase data is wrapped within this small range. One of the more reliant solutions of phase unambiguity is phase unwrapping, where the it is unwrapped based on the surrounding data. Unwrapping, however, works best on continuous phase changes and fails when abrupt phase jumps appear, e.g steps greater than the unambiguous range. An alternative method to overcome the unambiguous phase problem is multiplexing two wavelengths to capture the same phase image. Processing together the two phase images captured by different wavelengths can produce a new image, corresponding to a phase image with a synthetic wavelength that larger than each of the original wavelengths. This effectively enlarges the unambiguous range, making costly unwrapping algorithms redundant.

We present a new dual-wavelength interferometry setup that can capture and multiplex two different phase images in an external module, portable to existing microscopy systems. The module is based on a self-interference multiplexing technique. As such, it is very flexible and can work with either transmission or reflection based microscopes. It can be used for either enlarging the unambiguous range or other dual-wavelength phase imaging applications.

10074-21, Session 4

Structured illumination for 3D reconstruction of refractive-index and fluorescence

Shwetadwip Chowdhury, Joseph A. Izatt, Duke Univ. (United States)

Refractive-index (RI) is an inherent optical property of materials that can provide important biochemical and biophysical information about a biological sample. Optical-diffraction-tomography (ODT) is a current standard to obtain quantitative three-dimensional RI distributions, by measuring optical fields diffracted from the sample by rotated illumination beams. This method for ODT also synthetically enlarges the microscope's lateral spatial-frequency support, and thus images the RI distribution with lateral resolution beyond the microscope's coherent diffraction limit. Fluorescence microscopy offers a complementary set of biological insights by offering imaging capabilities with molecular specificity. Analogous to ODT, super-resolution fluorescence techniques can offer these insights at spatial resolutions beyond the microscope's incoherent diffraction limit. Unfortunately, such super-resolution techniques are generally incompatible with ODT and a generalized sub-diffraction technique has been difficult to find, which hinders a cohesive, high-resolution, multimodal analysis of

biological samples. We experimentally introduce, for the first time to our knowledge, a novel, high resolution, optical system that uses structured illumination (SI) to enable 3D sub-diffraction resolution imaging for both fluorescence and RI. We demonstrate sub-diffraction resolution, multimodal SI imaging of HT29 and MCF7 cells fluorescently stained for F-actin, such that the 3D RI and fluorescent distributions may offer unique, but complementary, insights into the biological samples.

10074-22, Session 4

Fluorescence exclusion: A simple versatile technique to decouple the height and the refractive index in QPI

Olivier Thouvenin, Mathias Fink, A. Claude Boccara, Institut Langevin (France)

Understanding volume regulation during mitosis is technically challenging. Indeed, a very sensitive non invasive imaging over time scales ranging from seconds to hours and over large fields is required. Therefore, Quantitative Phase Imaging (QPI) would be a perfect tool for such a project. However, because of asymmetric protein segregation during mitosis, an efficient separation of the refractive index and the height in the phase signal is required.

Even though many strategies to make such a separation have been developed, they usually are difficult to implement, have poor sensitivity, or cannot be performed in living cells, or in a single shot.

In this paper, we will discuss the use of a new technique called fluorescence exclusion to perform volume measurements. By coupling such technique with a simultaneous phase measurement, we were also able to recover the refractive index inside the cells.

Fluorescence exclusion is a versatile and powerful technique that allows the volume measurement of many types of cells. A fluorescent dye, which cannot penetrate inside the cells, is mixed with the external medium in a confined environment. Therefore, the fluorescent signal depends on the inverse of the object's height. We could demonstrate both experimentally and theoretically that fluorescence exclusion can accurately measure cell volumes, even for cells much higher than the depth of focus of the objective. A local accurate height and RI measurement can also be obtained for smaller cells. We will also discuss the way to optimize the confinement of the observation chamber, either mechanically or optically.

10074-23, Session 4

Quantitative differential phase contrast imaging by asymmetric pupil modulation

Hangwen Lu, Jaebum Chung, Xiaoze Ou, Changhuei Yang, California Institute of Technology (United States)

Differential phase contrast (DPC) is a non-interference quantitative phase imaging method achieved by asymmetric optical systems. Quantitative DPC images are achieved previously with asymmetric illumination systems. However, it works well for on-focus thin samples only. Considering the limitation, we develop a pupil modulation differential phase contrast (PMDPC) imaging method. Instead of modulating the illumination, we use a spatial light modulator (SLM) to modulate a 4f imaging system's pupil plane. When half of the pupil plane is blocked by the SLM, a phase gradient image forms on the image plane. Using two such phase gradient images captured separately by applying complementary half-circle pupils on SLM, a DPC image can be constructed that carries the sample's phase information. A quantitative phase image of the sample can be reconstructed after a deconvolution procedure. Further, we are able to combine this quantitative phase with the sample's intensity image to obtain the complete complex object field which then allows us to post-process the image. We report experimentally that aberrations arising from the optical elements in the system can be corrected by deconvolving the reconstructed image with a pre-calibrated pupil function. We can also digitally extend the depth of field using angular spectrum propagation algorithm. With our PMDPC imaging setup where NA equals to 0.36, a quantitative phase image with periodic resolution of 1.73μm is obtained. The depth of field for a 20x, 0.4NA objective is extended digitally by 20 times to ~50~50 micrometers.

10074-24, Session 4

Photothermal quantitative phase imaging of living cells with nanoparticles utilizing a cost-efficient setup

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In photothermal quantitative phase imaging (PTQPI) cells are incubated with functionalized nanoparticles (NPs) that bind to specific target structures to achieve molecular specificity. Excitation of the NPs with laser light induces a photothermal signal. The resulting optical path length changes can be detected by quantitative phase imaging. Compared to other techniques, e.g., fluorescence imaging, the method allows simultaneous acquisition of global cell parameters, like thickness, refractive index or dry mass, and molecule specific information about the target structures with the same experimental setup during a single measurement. Typically, PTQPI is performed with high excitation frequencies. This requires specific equipment like fast image recording devices that can be expensive. We explored PTQPI with a cost-efficient setup on the basis of a modified cell culture microscope that is suitable for usage in a biomedical environment. The excitation light was modulated by a mechanical chopper wheel with low frequencies, while quantitative phase imaging was performed with Michelson interferometer-based digital holographic microscopy. For off-axis digital holographic microscopy acquisition, a standard industrial camera was used. We present results from PTQPI observations on breast cancer cells, which were incubated with functionalized gold nanoparticles that bind to the epidermal growth factor receptor. Moreover, quantitative phase imaging was used to quantify the impact of NPs and the low frequency light excitation on cell morphology and viability.

10074-25, Session 4

Simultaneous imaging of quantitative phase and fluorescences using single-pixel detectors

Patrick A. Stockton, Colorado State Univ. (United States)

We present a novel single-pixel imaging technique that simultaneously images fluorescence and quantitative phase of an object. To extract simultaneously co-registered fluorescence and phase images, the object is illuminated by a pair of spatially coherent monochromatic laser beams with a difference in illumination spatial frequency that is swept linearly in time. One of the beams is stationary – serving as a reference beam – and propagated along the optic axis. The other beam scans through the full range of transverse spatial frequencies supported by the illumination optic – sweeping the crossing angle of the two beams incident on the specimen as a function of time. The scanned beam also has a temporal modulation carrier frequency that allows the extraction of the products of interfering fields. To record a phase image, forward scattered light from a thin object is collected in the back Fourier plane of a collection optic. Placing a narrow slit in the back Fourier plane allows the complex spatial frequency spectrum of the object amplitude transmission to be recorded in time. At each time point, the spatial frequency value corresponding to the difference in transverse spatial frequency of the illumination beams is recorded. Simultaneously, the interference of the illumination beams in the object

imparts a spatial frequency pattern on the fluorescent molecule excitation and the spatial frequency of the object's fluorescent concentration is recorded at each time step. This single-pixel imaging method allows for simultaneous acquisition of the object phase and fluorescent images by collecting spatial frequency projections in time.

10074-26, Session 5

Phase imaging of mechanical properties of live cells *(Invited Paper)*

Adam Wax, Duke Univ. (United States)

The mechanisms by which cells respond to mechanical stimuli are essential for cell function yet not well understood. Many rheological tools have been developed to characterize cellular viscoelastic properties but these typically require direct mechanical contact, limiting their throughput. We have developed a new approach for characterizing the organization of subcellular structures using a label free, noncontact, single-shot phase imaging method that correlates to measured cellular mechanical stiffness. The new analysis approach measures refractive index variance and relates it to disorder strength. These measurements are compared to cellular stiffness, measured using the same imaging tool to visualize nanoscale responses to flow shear stimulus. The utility of the technique is shown by comparing shear stiffness and phase disorder strength across five cellular populations with varying mechanical properties. An inverse relationship between disorder strength and shear stiffness is shown, suggesting that cell mechanical properties can be assessed in a format amenable to high throughput studies using this novel, non-contact technique. Further studies will be presented which include examination of mechanical stiffness in early carcinogenic events and investigation of the role of specific cellular structural proteins in mechanotransduction.

10074-27, Session 5

High resolution spatial coherence tomography for quantitative phase imaging of human red blood cells

Vishal Srivastava, Thapar Univ. (India); Azeem Ahmad, Dalip S. Mehta, Indian Institute of Technology Delhi (India)

Recently, there has been lot of development in the field of quantitative phase microscopy which, provides the quantitative information such as structure, morphology and composition and that can be obtain from phase-based analysis. Most of the conventional quantitative phase microscope systems are based on low coherence interferometry, in which a spatially highly coherent and temporally low coherent or spectrally broad band light source (white light) are used to obtain high axial resolution images. Therefore, the broader band light source is used to obtain a high axial resolution. The main disadvantage of broad band light source i.e., low temporally coherent requires dispersion compensation optics and high spatial coherence requires speckle reduction mechanism. We developed a full-field spatial coherence tomography microscope using temporally highly coherent (monochromatic) and spatially incoherent light source. As we know that birefringence of a material is wavelength dependent and the birefringence of RBC is a vital parameter for determining the average cell mass, thickness and haemoglobin concentration etc. We record the five phase shifted interferogram by a FF-SCT microscope for red and green colour wavelength and processed it to find out the birefringence of RBC for red and green colour wavelength. The present method is very useful for obtaining the high resolution images without dispersion effects and gives true colour spectroscopy. The principle, experimental details and results for human RBC, and will be later presented.

10074-28, Session 5

Quantitative assessment of cancer cell morphology and movement using telecentric digital holographic microscopy

Thanh C. Nguyen, Georges Nehmetallah, Van Lam, Byung Min Chung, Christopher B. Raub, The Catholic Univ. of America (United States)

Digital holographic microscopy (DHM) provides label-free and real-time quantitative phase information relevant to the analysis of dynamic biological systems. A DHM based on telecentric configuration optically mitigates phase aberrations due to the microscope objective and linear high frequency fringes due to the reference beam thus minimizing digital aberration correction needed for distortion free 3D reconstruction. The purpose of this work is to quantitatively assess growth and migratory behavior of invasive cancer cells using a telecentric DHM system. Together, the height and spread area of individual cells, determined from time-lapse series of phase reconstructions, should reveal morphological features controlled by cell-cell and cell-matrix adhesions. To test this, invasive MDA-MB-231 breast cancer cells were cultured on collagen-coated or un-coated glass, and 3D holograms were reconstructed over 2 hours with the DHM system. Obtained results show that cells on collagen-coated glass had ~an average 14% larger spread area than cells on uncoated glass (n=18-22 cells/group). Pre-mitotic cell rounding was observed with average phase height increasing 57% over ~10 minutes. Following cell division phase height decreased linearly (R2=0.94) to ~58% of the original height pre-division. Phase objects consistent with lamellipodia were apparent from the reconstructions at the leading edge of migrating cells. These data demonstrate the ability to track quantitative phase parameters and relate them to cell morphology during cell migration and division on adherent substrates, using telecentric DHM. The system is sensitive to cell shape changes, thus enabling future studies of cell-matrix interactions relevant to the role of biomechanics in cancer.

10074-29, Session 5

Monitoring of live cell cultures during apoptosis by phase imaging and Raman spectroscopy

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Non-invasive live cell measurements are an important tool in biomedical research. We present a combined digital holography/Raman spectroscopy technique to study live cell cultures during apoptosis. Digital holographic microscopy records an interference pattern between object and reference waves, so that the computationally reconstructed holographic image contains both amplitude and phase information about the sample. When the phase is mapped across the sample and converted into height information for each pixel, a three dimensional image is obtained. The measurement of live cell cultures by digital holographic microscopy yields information about cell cycle and cell death mechanisms, since these processes are correlated with individual cell volume and shape. Raman spectroscopy, on the other hand, is sensitive to rotational and vibrational molecular transitions, as well as intermolecular vibrations. Therefore, Raman spectroscopy provides complementary information about cells, such as protein, lipid and nucleic acid content, and, particularly, the spectral signatures associated with structural changes in molecules. The cell cultures are kept in the temperature-controlled environmental chamber during the experiment, which allows monitoring over multiple cell cycles. The DHM system combines a visible (red) laser source with conventional microscope base, and LabVIEW-run data processing. The Raman system uses a fiber probe to remotely collect the data from the cell samples. We analyzed and compared cell culture information obtained by these two methods.

10074-30, Session 5

Quantitative phase imaging of mitotic cells upon Noc treatment using d'Biomager to govern cell fate

Ke Guo, Rosmin E. Mohan, Nanyang Technological Univ. (Singapore); Ruiqi R. Wang, d'Optron Pte Ltd. (Singapore); Karen C. Crasta, Anand K. Asundi, Nanyang Technological Univ. (Singapore)

A general procedure involving defocus-based methods involves moving the sample (or camera) axially while capturing multiple images through–focus. This requires mechanical moving parts and thus does not allow for fast process to be captured. The d'Biomager makes use of tunable optics to circumvent this problem. It comprises a 4f optical configuration (50mm FL achromatic lenses) and a tuneable lens (Optotune EL-10-30) in the centre allowing to record images around focus in the image plane. The illumination is a collimated LED operating at a center wavelength of 633 nm. The application of current in the lens changes the optical power and thus the image plane position. Estimation of the intensity derivative is performed using finite difference method by measuring the intensity at different planes (at least two) around the focus. The phase can be then determined that enables one to make Z-axis thickness measurements and obtain accurate 3D images. Additionally, the use of a tunable lens (electrically monitored) allows the system to be capable for real time imaging at millisecond temporal resolution. The focus and defocus images are used to estimate dI/dz based on a current-distance calibration curve. The phase can thus be obtained from the Optical Path Difference for a known refractive index. We have used four cell lines in our study: HeLa, human cervix adenocarcinoma cell line which is prior to mitotic cell death during prolonged mitotic arrest caused by Noc; hTERT-RPE-1(immortalized human retinal pigment epithelial cell line) and U2OS (human bone osteosarcoma cell line) in which majority will undergo slippage post Noc treatment; U2OS-shATG5, generated from U2OS by transducing shATG5 to inhibit autophagy. The various phenotypes are captured and analyzed such as cell blebbing during prolonged mitotic (either undergoing apoptosis or mitotic slippage), and cell fates post slippage (either arrest in pseudo-G1 as multinucleated cells or die post slippage). The cell fates are investigated upon treatment with anti-mitotic drugs such as commonly used microtubule poison-Nocodazole (Noc). The cells are tracked individually in a time-lapse of 24 hours since adding Noc. The phase thus retrieved using the d'Biomager is simulated as a stack to animate cell behaviour. The novel optics and algorithm allows for near real-time measurement of phase which can further add clarity to the mitotic stage.

10074-31, Session 5

Applications of holographic on-chip microscopy *(Invited Paper)*

Aydogan Ozcan, Univ. of California, Los Angeles (United States)

My research focuses on the use of computation/algorithms to create new optical microscopy, sensing, and diagnostic techniques, significantly improving existing tools for probing micro- and nano-objects while also simplifying the designs of these analysis tools. In this presentation, I will introduce a set of computational microscopes which use lens-free on-chip imaging to replace traditional lenses with holographic reconstruction algorithms. Basically, 3D images of specimens are reconstructed from their "shadows" providing considerably improved field-of-view (FOV) and depth-of-field, thus enabling large sample volumes to be rapidly imaged, even at nanoscale. These new computational microscopes routinely generate >1–2 billion pixels (giga-pixels), where even single viruses can be detected with a FOV that is >100 fold wider than other techniques. At the heart of this leapfrog performance lie self-assembled liquid nano-lenses that are computationally imaged on a chip. The field-of-view of these computational microscopes is equal to the active-area of the sensor-array, easily reaching, for example, >20 mm^2 or >10 cm^2 by employing state-of-the-art CMOS or CCD imaging chips, respectively.

In addition to this remarkable increase in throughput, another major benefit of this technology is that it lends itself to field-portable and cost-effective designs which easily integrate with smartphones to conduct giga-pixel tele-pathology and microscopy even in resource-poor and remote settings where traditional techniques are difficult to implement and sustain, thus opening the door to various telemedicine applications in global health. Through the development of similar computational imagers, I will also report the discovery of new 3D swimming patterns observed in human and animal sperm. One of this newly discovered and extremely rare motion is in the form of "chiral ribbons" where the planar swings of the sperm head occur on an osculating plane creating in some cases a helical ribbon and in some others a twisted ribbon. Shedding light onto the statistics and biophysics of various micro-swimmers' 3D motion, these results provide an important example of how biomedical imaging significantly benefits from emerging computational algorithms/theories, revolutionizing existing tools for observing various micro- and nano-scale phenomena in innovative, high-throughput, and yet cost-effective ways.

10074-32, Session 5

Non-contact measurement of electrical activity in neurons using magnified image spatial spectrum (MISS) microscopy

Hassaan Majeed, Young Jae Lee, Catherine Best-Popescu, Gabriel Popescu, Sung-Soo Jang, Hee Jung Chung, Univ. of Illinois at Urbana-Champaign (United States)

Traditionally the measurement of electrical activity in neurons has been carried out using microelectrode arrays that require the conducting elements to be in contact with the neuronal network. This method, also referred to as "electrophysiology", while being excellent in terms of temporal resolution is limited in spatial resolution and is invasive. An optical microscopy method for measuring electrical activity is thus highly desired. Common-path quantitative phase imaging (QPI) systems are good candidates for such investigations as they provide high sensitivity (on the order of nanometers) to the plasma membrane fluctuations that can be linked to electrical activity in a neuronal circuit. In this work we measured electrical activity in a culture of rat cortical neurons using MISS microscopy, a high-speed common-path QPI technique having an axial resolution of around 1 nm in optical path-length, which we introduced at PW BIOS 2016. Specifically, we measured the vesicular cycling (endocytosis and exocytosis) occurring at axon terminals of the neurons due to electrical activity caused by adding a high K+ solution to the cell culture. The axon terminals were localized using a micro-fluidic device that separated them from the rest of the culture. Stacks of images of these terminals were acquired at 826 fps both before and after K+ excitation and the temporal standard deviation maps for the two cases were compared to measure the membrane fluctuations. Concurrently, the existence of vesicular cycling was confirmed through fluorescent tagging and imaging of the vesicles at and around the axon terminals.

10074-33, Session 5

Quantifying collagen orientation in breast tissue biopsies using spatial light interference microscopy (SLIM)

Hassaan Majeed, Chukwuemeka Okoro, Univ. of Illinois at Urbana-Champaign (United States); Andre Balla, Univ. of Illinois at Chicago (United States); Kimani C. Toussaint Jr., Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Breast cancer is a major public health problem worldwide, being the most

common type of cancer among women according to the World Health Organization (WHO). The WHO has further stressed the importance of an early determination of the disease course through prognostic markers. Recent studies have shown that the alignment of collagen fibers in tumor adjacent stroma correlate with poorer health outcomes in patients. Such studies have typically been carried out using Second-Harmonic Generation (SHG) microscopy. SHG images are very useful for quantifying collagen fiber orientation due their specificity to non-centrosymmetric structures in tissue, leading to high contrast in collagen rich areas. However, the imaging throughput in SHG microscopy is limited by its point scanning geometry. In this work, we show that SLIM, a wide-field high-throughput QPI technique, can be used to obtain the same information on collagen fiber orientation as is obtainable through SHG microscopy. We imaged a tissue microarray containing both benign and malignant cores using both SHG microscopy and SLIM. The cellular (non-collagenous) structures in the SLIM images were next segmented out using an algorithm developed in-house. Using the previously published Fourier Transform Second Harmonic Generation (FT-SHG) tool, the fiber orientations in SHG and segmented SLIM images were then quantified. The resulting histograms of fiber orientation angles showed that both SHG and SLIM generate similar measurements of collagen fiber orientation. The SLIM modality, however, can generate these results at much higher throughput due to its wide-field, whole-slide scanning capabilities.

10074-34, Session 5

Micro patterned surfaces: an effective tool for long term digital holographic microscopy cell imaging

Sarah Mues, Westfälische Wilhelms-Univ. Münster (Germany); Inga Lilge, Holger Schönherr, Univ. Siegen (Germany); Björn Kemper, Jürgen Schnekenburger, Westfälische Wilhelms-Univ. Münster (Germany)

Digital holographic microscopy is a powerful tool for multimodal long-term cell imaging. Cellular processes as proliferation, migration or cell death can be recorded online from minutes up to several days. The technology is applied to the measurement of cellular effects induced by toxic agents, drug or specifically changed protein expression. Using automated cell tracking software the technology can detect and analyze large panels of cells in parallel. The simple but major problem of the approach is the fact that over time most of the tracked cells move out of the image area. Therefore most of the cells are lost for the evaluation of cellular processes. We here present an effective solution for this crucial problem of long-term microscopical live cell analysis. We have generated functionalized glass slides containing 100 x 150 μm areas for selective cell adhesion surrounded by hydrophobic areas preventing cell adhesion. The structures were produced by PDMS-stamp micro contact printing followed by the polymerization of a 50 nm thick PAAm brush film and a backfill with hydrophobic layers. These cages designed for the size our microscopic image areas were highly effective in keeping all cells inside the rectangles over the selected imaging period. We have successfully used these microstructures with different cell types for up to 72 h. Cell move and grow sharply to the edges of the areas but will not leave the structures. These highly effective micropatterned surfces are ideal substrates for long term imaging of various cellular process.

10074-53, Session PMon

Low cost label-free live cell imaging for biological samples

Chandrabhan Seniya, Catherine E. Towers, David P. Towers, The Univ. of Warwick (United Kingdom)

This paper reports the progress to develop a practical phase measuring microscope offering new capabilities in terms of phase measurement accuracy and quantification of cell:cell interactions over the longer term. A novel, low cost, phase shifting microscope for label-free live cell imaging is described. The method combines the Zernike phase contrast approach with a dual mirror design to enable phase modulation between the scattered and un-scattered optical fields. The design retains the common path nature of Zernike's concept and the phase shift is simple to control via a piezoelectric driven mirror in the back focal plane of the imaging system. The approach is significantly cheaper to implement than those based on spatial light modulators (SLM) at approximately 20% of the cost.

The microscope enables different phase shifting algorithms to be explored with varying source bandwidths, where it is widely accepted that increasing the bandwidth of the optical source reduces noise in the interferograms obtained [1]. A study has been conducted to explore the effect of the phase shifting algorithm on the accuracy of the calculated phase for samples corresponding to several layers of cells. The results indicate that either the 5-frame at 90 degree steps [2] or 6+1-frame at 60 degree steps [3] algorithms offer improved phase reconstruction accuracy. Data will be presented showing algorithm performance under different conditions.

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10074-54, Session PMon

MTF evaluation of in-line phase contrast imaging system

Xiaoran Sun, Feng Gao, Huijuan Zhao, Limin Zhang, Jiao Li, Zhongxing Zhou, Tianjin Univ. (China)

X-ray Phase contrast imaging(XPCI) is a novel method that exploits the phase shift for the incident X-ray to form an image. Various XPCI methods has been proposed, among which, in-line phase contrast imaging is regarded as one of the most promising clinical method owing to simple setups and high stability.The current simulation of in line phase contrast imaging is generally analytic simulations based on Fresnel–Kirchhoff wave-front diffraction formulation. However, analytic simulations are not appropriate for nonideal imaging system and complex experimental conditions, such as first-order or higher-order scattering, objects with complex geometry, image degradation. In this work, a Monte Carlo method is employed to obtain accurate phase contrast images under complex conditions of imaging system, including finite-size-source effect, beam divergence, pixel type and detector orientation. The results show that we can achieve accurate simulation of multispectral in-line phase contrast imaging and explore the relationship between system parameters and the quality of phase contrast image.

10074-55, Session PMon

Phase retrieval for non-ideal in-line phase contrast x-ray imaging

Baikuan Guo, Feng Gao, Huijuan Zhao, Limin Zhang, Jiao Li, Zhongxing Zhou, Tianjin Univ. (China)

Phase contrast X-ray imaging techniques have shown the ability to overcome the weakness of the low sensitivity of conventional X-ray imaging. Among which, in-line phase contrast imaging, blessed with simplicity of arrangement, is deemed to be a promising technique in clinical application. To obtain quantitative information from in-line phase contrast images, numerous phase retrieval techniques have been developed. The theories of these phase retrieval techniques are mostly proposed on the basis of ideal x-ray source and detector. However, in practice, the point spread function of the the X-ray source and detector degrades the effect of phase contrast. To alleviate such negative influence, a deconvolution technique is incorporated into the phase retrieval algorithm. The results show that the proposed technique improves the accuracy of phase retrieval under the condition of more readily available x-ray sources and detectors.

10074-56, Session PMon

Dual-wavelength optical diffraction tomography utilizing a digital micromirror device

JaeHwang Jung, YoungKeun Park, KAIST (Korea, Republic of)

To enhance the molecular sensitivity of quantitative phase imaging (QPI), spectroscopic imaging has been one of the emerging application of QPI. Because every molecule has own distinct spectroscopic properties including absorption and refractive index, spectroscopic properties are considered as crucial information in identification, quantification, and separation of chemical and biological components. Although several studies presented spectroscopic 2-dimensional QPI techniques, demonstration of spectroscopic 3-dimensional (3-D) QPI has been limited due to the complexity of an optical system and limited application.

Here we present simultaneous measurements of the 3-D complex refractive index distribution of microscopic samples at two different wavelengths using optical diffraction tomography (ODT). The optical configuration of this ODT system is based on Mach-Zehnder interferometry equipping a digital micromirror device (DMD) to change incident angles. To illuminate a sample at two wavelengths, two coherent lasers of different wavelengths are coupled into 2?2 fiber couplers at each an input port. One output is directed to the DMD to illuminate a sample at various angles, and another output is used as a reference arm. The field information of two co-aligned lights is decoupled by using a color camera. To verify a capability of the system, we measure microscopic green alga, Chlorella Vulgaris, and quantify an amount of chlorophyll inside a single individual Chlorella. Because chlorophyll a and chlorophyll b have different absorption spectra and refractive index dispersion, we could identify the location and quantify the amount of chlorophylls inside Chlorella.

10074-57, Session PMon

Measurements of polarization-dependent 2D angle-resolved light scattering of individual microscopic objects

JaeHwang Jung, YongKeun Park, KAIST (Korea, Republic of)

Employing polarizer-equipped quantitative phase microscopy and a Fourier transform light scattering technique, the complex scattered fields were quantitatively measured for four combinations of polarization directions of incident and scattered fields. We demonstrated the technique on a silver nanowire and a liquid crystal droplet which presents strong geometrical anisotropy and optical anisotropy, respectively. Four-component measurements of the scattering field will provide a novel experimental approach for understanding polarization dependent scattering from microscopic objects.

10074-58, Session PMon

Label-free quantitative investigation of microfluidic mixing using quantitative phase imaging

GwangSik Park, Dongsik Han, GwangSu Kim, Kyoohyun Kim, Je-Kyun Park, YongKeun Park, KAIST (Korea, Republic of)

Microfluidic devices provide a highly controllable environment for various research fields including fluidic dynamics, high throughput cell screening, and analytical chemistry. Among various dynamic processes taking places in microfluidic channels, microfluidic mixing of different liquids is one of the interesting phenomena which exhibits distinctive aspects of fluid dynamics depending on several fluid conditions including the viscosity, density, and velocity of input liquids and the channel geometry. To investigate the dynamics of microfluidic mixing, conventionally fluorescence imaging has been utilized where the emitted light from different fluorescent dyes dissolved in liquids is measured. However, fluorescence imaging has limited access for quantitative analysis of microfluidic mixing since the intensity of fluorescent dyes is not directly proportional to the concentration of liquids.

Here, we present an interferometric method to quantitatively analyze microfluidic mixing. A Mach-Zehnder interferometer measures optical phase delay of mixing liquids inside microfluidic channels. Since the size of microfluidic channels extends the field of view of detecting optics, optical phase delay images of successive regions of microfluidic channels were stitched. From measured optical phase delay, the concentration of mixing liquids was successfully calculated because the refractive index values of liquids are proportional to liquid concentration. We demonstrate the quantitative analysis of microfluidic mixing inside microfluidic channels having different geometry.

10074-59, Session PMon

Remotely sensing pressure inside microchannels using light scattering in Scotch tape

KyungDuk Kim, Hyeonseung Yu, Joon-Young Koh, Jung H. Shin, Won-Hee Lee, YongKeun Park, KAIST (Korea, Republic of)

Fluidic flows in microchannels play an important role for the efficient transport of matter or energy in microelectromechanical systems. A variety of gaseous systems has been miniaturized into microchannel systems, such as a gas analyzer and a cooling system. In the study of gas flows in microchannels, it is important to measure the pressure inside channels for characterizing or controlling the gaseous environment. To directly measure the pressure inside the channel, external transducers and micro-fabricated built-in sensors were proposed, but these methods are expensive and provide low-resolution. Various optical methods to measure pressure also have been proposed, such systems based on optical fiber sensors, Mach–Zehnder interferometry, Fabry–Perot etalon, and fiber Bragg grating. They can provide high sensitivity of detecting the pressure-induced deformation in order of a wavelength. However, the difficulty of integrating them with the channel of a small size restricts the practical use. Here we propose a simple method to measure the pressure inside a deformable microchannel using light scattering in an opaque Scotch tape. When a coherent laser beam passes through the channel and the Scotch tape, the speckle patterns are formed by light scattering in Scotch tape. The resultant speckle patterns are highly sensitive to the minute change of the wavefront due to the randomness of scattering layer. By measuring the speckle patterns, the deformation of the channel induced by the internal pressure change is effectively measured. We demonstrate that with a proper calibration, internal pressure can be sensed with a resolution of 0.1 kPa within 0–3 kPa.

10074-60, Session PMon

Anisotropy imaging using polarization and angular multiplexing

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Anisotropy of the sample plays significant role in areas of cell biology, biophysics, biomedical imaging, chemistry, mineralogy and found

applications in imaging, diagnosis, spectroscopy, sensing etc. Majority of the studies on the subject are based on extraction of anisotropic features by employing Jones, or Mueller matirx formalisms. Jones matrix formalism characterizes the vectorial transmission properties of the sample by coherent approach using 2?2 complex matrix elements. The recent advancements in Jones matrix imaging techniques change its complexity from a four step process to a single step process. Different techniques have been proposed in recent years to image complex Jones matrix elements of the sample.

In this paper, we propose a new strategy for measurement of anisotropy using Jones matrix elements by making use of polarization interferometer with angular multiplexing. An interferometric approach equipped with polarizing interferometers in both object and reference arm provides the required angular multiplexing. The principle of the technique lies in the simultaneous illumination of the sample with +45 and -45 linearly polarized beam and subsequent retrieval of Jones matrix elements from a recorded interference pattern. The technique offers retrieval of all elements of the Jones matrix from a single measurement. The basic principle of the technique is described and as a proof of the proposed imaging strategy, preliminary results with known samples are presented.

10074-61, Session PMon

Single-shot digital holographic microscopy for extracting spatially resolved Jones matrix

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Polarization of light is an intrinsic property representing the direction of rotational momentum of the light. The polarization is very sensitive to the optical anisotropy of an object and thus can effectively visualize the structure with birefringence of a target sample. Due to the complex nature of the polarization, the digital holographic imaging methods have been utilized for describing the polarization properties of an object quantitatively. For the complete description of the polarization response of an object, four independent measurements are required to construct a 2-by-2 matrix, so called Jones Matrix, according to the two orthogonal measurement axes.

Here we demonstrate digital holographic microscopy for extracting spatially resolved Jones Matrix of an object with a single-shot measurement. Four different copies of an object image are generated by the imaging splitting method using a two-dimensional diffraction grating. With a separate reference beam of an off-axis configuration, the complex field images for all the copies are obtained. By introducing polarization filters with four different combinations for the image copies, four elements of the complete Jones Matrix can be acquired at a time. With this method, the anisotropic structures of biological samples with relatively higher birefringence, such as river tissues and rat tendons, can be visualized. This method will facilitate the measurement of fast dynamics of polarization properties of the samples.

10074-62, Session PMon

Optical characterization of tissue-simulating phantoms with microparticles by digital image plane holography

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Digital Image Plane Holography is a non-invasive optical technique which is able to recover the whole object wave. An object is illuminated and the diffused backscattered light is carried to a digital sensor by using a lens, where it interferes with a divergent reference wave with its origin in the lens aperture plane. Selecting each aperture image in the Fourier plane, the amplitude and the phase of the object beam are obtained. If two holograms are recorded at different times, after a small displacement, the reconstructed intensity distributions can be taken as a speckle field, while the phase difference distribution can be analyzed by an interferometric approach.

In this work biological tissue phantoms are investigated by using digital holography. The aim of this paper is to determine the viability of the technique to characterize optical properties of scattering samples. Biological tissue phantoms are made of microparticles. Different phantoms generate a speckle pattern with different statistical properties (size, contrast, intensity). Both the visibility of the interferometric fringes and the properties of speckle pattern are related with optical properties of the media such as absorption and scattering coefficients. The ability to measure these properties makes the technique a promising method for biomedical applications.

10074-63, Session PMon

Low cost production of a versatile 3D-printed perfusion chamber for quantitative phase imaging of primary neurons in culture

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Quantitative phase imaging (QPI) allows quantifying the phase shift (PS) introduced by a specimen. PS arises from the difference in refractive index (RI) between the specimen and the surrounding medium and is proportional to the thickness of the observed transparent sample. Because of this duality, QPI signal remains difficult to interpret. In cellular imaging, there are two types of approaches regarding QPI. Firstly, the user seeks to extract the RI and the sample thickness independently. Accordingly, some decoupling procedures have been developed to separately measure cell thickness and RI. Secondly, the PS can be used as a biologically meaningful quantity in electrophysiological studies. However, both types of experiment require fundamentally different microscopy accessories and protocols. We developed a low cost and versatile 3D-printed perfusion chamber for QPI of primary neurons in culture. First, the cell chamber is 3D-printed in biocompatible plastic. The chamber is easily convertible between a closed configuration, for decoupling, and an open arrangement, for electrophysiology. In the closed system, the imaging volume is small, allowing a rapid laminar flow with fast turnover from the standard perfusion solution to a second medium with a different RI. In the open structure, the field of view is large, to visualize neurons network, and the profile of the chamber is shallow, permitting the access of pipettes to neurons. The chamber we built is therefore tailored to fit the specific needs of QPI. Finally, we report successful decoupling an electrophysiology experiments on neurons during an osmotic choc.

10074-64, Session PMon

The study on the parallel processing based time series correlation analysis of RBC membrane flickering in quantitative phase imaging

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Quantitative phase imaging (QPI) can provide the subnanometer optical path length sensitivity of the transparent object within a millisecond time

scale. RBC has flexible membrane structure and thermal affected membrane fluctuations. The mechanical information of RBC membrane fluctuations using QPI has been presented for the five years. QPI based time series correlation analysis of RBC have been introduced for the precision blood diagnosis method. However, time series correlation analysis using QPI has required a time consuming process because of the large sample size and the complex computation. In this study, we present the improvement of quantitative phase imaging processing speed for rapid time series correlation analysis of RBC membrane fluctuations over the whole surface using heterogeneous parallel process computing. Time dependent thickness variation and RBC membrane is calculated and gathered data is processed by parallel processing algorithm. We have applied our proposed parallel processing method to Detrended Fluctuation Analysis (DFA) of RBC membrane fluctuations described by the previous study. Measured ? values of healthy RBC were distributed between 0.7 and 1.0 and calculated within 60 seconds over the whole surface of RBC.

10074-66, Session PMon

Cells and tissues refractive index investigation using the statistical dispersion relation (SDR)

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Refractive index (RI) governs the light motion interaction in cells and tissues and offers potential for label-free diagnosis. RI can be measured using quantitative phase imaging (QPI). But measuring the refractive index from a QPI image remains a great challenge because it requires knowledge of the tissue thickness. Here, we present a statistical dispersion relation (SDR) for waves propagating in inhomogeneous media, and use it with QPI experimental results to extract information about the refractive index distribution of cells and tissues without prior knowledge of thickness. The SDR works within the Born approximation, and incorporates the statistics of the wavevector distribution and that of the refractive index in the medium. Considering that the wavevector variance can be obtained from the measurable phase shift?we then connect to the refractive index variance with the experimental data. We employed spatial light interference microscopy (SLIM) to measure the phase shift maps for both cells and tissue slices. Experimental results show that refractive index variance of neurons, colon biopsy and papillary thyroid cancer can be revealed from QPI images through SDR. We anticipate that SDR will provide a wide range of applications, from basic cell studies to clinical application.

10074-67, Session PMon

Phase shifting white light diffraction phase microscopy (PSwDPM) for high space-bandwidth product QPI

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White light diffraction phase microscopy (wDPM) enables single shot measurement, high temporal phase stability, and high spatial phase sensitivity. However, due to its off-axis configuration, wDPM suffers from either lower transverse resolution, or smaller field of view when compared to phase shifting methods. The reduced space-band width product is characteristic to all off-axis methods. It is due to the image no longer being sampled by the camera pixels, but, instead by the interference spatial frequency. Phase-shifting methods remove this obstacle at the expense of time-bandwidth product. In this paper, we present a new method, referred to as phase shifting white light diffraction phase microscopy (PSwDPM), to improve quantitative phase imaging for biological studies. We implemented PSwDPM by introducing a phase shift of ϖ to the grating in wDPM, and then acquiring only 2 intensity images to obtain each quantitative phase image. Compared to wDPM, PSwDPM illustrates the tradeoffs between transverse resolution and acquisition rate. We demonstrate the utility of PSwDPM with measurements on USAF resolution target, polystyrene micro-bead, Hela cells and tissue slices.

10074-68, Session PMon

Dispersion-relation phase spectroscopy in 3D

Mikhail E. Kandel, Univ. of Illinois at Urbana-Champaign (United States); Alison M. Taylor, Univ. of Illinois at Urbana-Champaign (United States) and American Univ. (United States); Catherine A. Best-Popescu, Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Despite broad applications for medical research, the mechanisms behind neuron growth and repair are poorly understood in terms of mass flow rates. Traditionally cellular traffic is studied with fluorescence microscopy, often by tagging secretory proteins and with neuronal processes co-localizing by immunofluorescence staining after fixation. Such experiments often suffer from the typical difficulties of fluorescence experiments such photoxicity and photobleaching. Here we show how label-free quantitative phase imaging (QPI) and spatial light interference microscope (SLIM) can improve upon existing methods by enabling widefield tomographic acquisition of neuronal traffic. We employ the dispersion-relation phase spectroscopy (DPS) to the SLIM tomographic data and obtain, for the first time to our knowledge, intracellular transport in 3D.

10074-35, Session 6

Quantitative phase microscopy of live cells flowing in a micro-channel using flipping interferometry

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Measurements of biological cells during flow are highly important for medical diagnosis based on cell sorting. In the case of cell imaging during flow, very rapid image acquisition capabilities are required to enable fast cell flow for analyzing a sufficient number of cells. We present a new flipping interferometry (FI) module for simplified off-axis close-to-common-path interferometric phase microscopy. This wide-field off-axis interferometric module provides rapid quantitative phase microscopy of biological cells during flow in a microfluidic channel, with potential of integration into cell sorting devices. Various experimental demonstrations are presented.

10074-36, Session 6

Chemotaxis of cancer cells in three-dimensional environment monitored label-free by quantitative phase digital holographic microscopy

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Quantification of cell migration and motility as response to chemical stimuli

is important in various areas of life sciences and medicine, in particular for cancer research and the analysis of the influence of drugs. Here cell observations in 3D environments in-vitro are of particular interest as such arrangements allow a better simulation of in-vivo situations. We investigated the capabilities of digital holographic microscopy (DHM) for label-free quantification of chemotaxis in 3D assays. Fibro sarcoma cells were cultivated in a collagen matrix inside 3D chemotaxis chambers and observed by time-lapse imaging with a Mach-Zehnder interferometer-based setup for off-axis DHM. Chemotaxis was induced by serum concentration gradients in the cell culture medium. The obtained series of quantitative holographic phase images were analyzed by automated single cell tracking. The resulting migration trajectories were subsequently evaluated for motility and directness. The results were compared to data from complementary investigations on cells in 2D chemotaxis chambers as well as on collagen coated surfaces and uncoated substrates. Cells in 3D collagen migrated faster and more directed than cells on 2D surfaces. Moreover, lack of serum in the environment of the cell cultures induced increased cell motility in all experimental configurations. In conclusion, our results demonstrate DHM as a highly reliable and efficient tool for label-free quantification of chemotaxis in 2D and 3D environments.

10074-37, Session 6

Digital holographic microscopy overcomes the limitations of in vitro nanomaterial cytotoxicity testing

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In vitro cytotoxicity assessment of engineered nanomaterials commonly involves the measurement of different cellular endpoints as reactive oxygen species formation, cell viability or cell death. Usually these parameters are determined by optical readouts of enzymatically-converted substrates that are often affected by nanomaterial-assay interference.

For many nanomaterials, especially materials with a low toxicity level, the material assay interference inhibits the detection of regulatory relevant the low observed effect level (LOEL). Here we present Digital Holographic Microscopy (DHM) as a multimodal optical method, which overcomes the limitations of conventional in vitro assays. Using cell viability (WST-8) and cell death (LDH) assays as parameter we initially investigated the toxic effects of spherical (NM 300) and rod shaped (NM 302) silver nanomaterials with a matrix of four cell lines representing different organ functions. In addition, we applied DHM for multimodal label-free analysis of nanomaterial toxicity. Quantitative DHM phase images of cells were analyzed for refractive index, volume, density and dry mass. Silver spheres induced cytotoxic effects in all four examined cell lines compared to no toxicity of rods up to 10 µg/cm2. Furthermore, we could correlate these data to a decrease of the cellular refractive index after incubation with NM 300 as well as a decreased dry mass and surface area development indicating reduced cell viability and cell death. DHM allowed us to increase the concentration of silver nano rods to the LOEL concentration. These results demonstrate the potential of DHM as novel valuable label-free tool for the analysis nanomaterial toxicity.

10074-38, Session 6

Characterization of cardiomyocytes motion using quantitative phase imaging

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Beating heart cells, cardiomyocytes, are used in drug testing and have the potential for regenerative medicine. Currently their classification into atrial, nodal and ventricular subtypes is performed using destructive and tedious patch clamp measurements. We present a method for analyzing cardiomyocyte contraction cycles using diffraction phase microscopy, a fast quantitative phase imaging method based on off-axis common-path interferometry. The phase variation during the beating cycle can exceed 300 mrad in the most active regions, and is about 40 mrad on average. The phase noise is about 2 mrad per pixel, and it can be reduced by temporal averaging over multiple frames and spatial averaging over the cell. With a maximum acquisition rate exceeding 25,000 fps and with approximately 100 fps required for the characterization of cardiomyocyte motion, 250 frames can be averaged per step, reducing the temporally white noise by a factor of 16. Additional improvements can be obtained by averaging over multiple contraction cycles. Averaging over space does not reduce noise to the same extent due to low-pass spatial filtering during the phase extraction procedure. Low-pass filtering by the pinhole in the reference arm, resulting in high-pass filtering of the image, and low-pass filtering during the phase reconstruction highlight the dynamics of redistribution of dry mass within the cell during a beat cycle. Quantitative phase imaging is a promising approach for rapid, non-invasive, high-throughput characterization of human stem cell-derived cardiomyocytes in culture, with applications to modeling of diseases with patients' specific genes, drug development, and repair of damaged heart tissue.

10074-39, Session 6

Three-dimensional refractive index and fluorescence tomography using structured illumination

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Optical diffraction tomography (ODT) has been an emerging optical technique for label-free imaging of three-dimensional (3-D) refractive index (RI) distribution of biological samples. ODT employs interferometric microscopy for measuring multiple holograms of samples with various incident angles, from which the Fourier diffraction theorem reconstructs the 3-D RI distribution of samples from retrieved complex optical fields. Since the RI value is linearly proportional to the protein concentration of biological samples where the proportional coefficient is called as refractive index increment (RII), reconstructed 3-D RI tomograms provide precise structural and biochemical information of individual biological samples. Because most proteins have similar RII value, however, ODT has limited molecular specificity, especially for imaging eukaryotic cells having various types of proteins and subcellular organelles.

Here, we present an ODT system combined with structured illumination microscopy which can measure the 3-D RI distribution of biological samples as well as 3-D super-resolution fluorescent images in the same optical setup. A digital micromirror device (DMD) controls the incident angle of the illumination beam for tomogram reconstruction, and the same DMD modulates the structured illumination pattern of the excitation beam for super-resolution fluorescent imaging. We first validate the proposed method for simultaneous optical diffraction tomographic imaging and super-resolution fluorescent imaging of fluorescent beads. The proposed method is also exploited for various biological samples.

10074-40, Session 7

Improved cancer risk stratification and diagnosis via quantitative phase microscopy *(Invited Paper)*

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Pathology remains the gold standard for cancer diagnosis and in some cases prognosis, in which trained pathologists examine abnormality in

tissue architecture and cell morphology characteristic of cancer cells with a bright-field microscope. The limited resolution of conventional microscope can result in intra-observer variation, missed early-stage cancers, and indeterminate cases that often result in unnecessary invasive procedures in the absence of cancer. Assessment of nanoscale structural characteristics via quantitative phase represents a promising strategy for identifying pre-cancerous or cancerous cells, due to its nanoscale sensitivity to optical path length, simple sample preparation (i.e., label-free) and low cost. I will present the development of quantitative phase microscopy system in transmission and reflection configuration to detect the structural changes in nuclear architecture, not be easily identifiable by conventional pathology. Specifically, we will present the use of transmission-mode quantitative phase imaging to improve diagnostic accuracy of urine cytology and the nuclear dry mass is progressively correlate with negative, atypical, suspicious and positive cytological diagnosis. In a second application, we will present the use of reflection-mode quantitative phase microscopy for depth-resolved nanoscale nuclear architecture mapping (nanoNAM) of clinically prepared formalin-fixed, paraffin-embedded tissue sections. We demonstrated that the quantitative phase microscopy system detects a gradual increase in the density alteration of nuclear architecture during malignant transformation in animal models of colon carcinogenesis and in human patients with ulcerative colitis, even in tissue that appears histologically normal according to pathologists. We evaluated the ability of nanoNAM to predict "future" cancer progression in patients with ulcerative colitis.

10074-41, Session 7

Opportunities of QPI in the epigenetic diagnostics and assessment of therapeutic efficacy

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Different specialists often noted the advantages of the interference microscopy methods: small invasiveness and presenting measurement results in rationed physical values (phase thickness and refractive indices – refractivity), and possibility to achieve a significant super-resolution of phase images and high speed of image registration. As a prospective investigation method, QPI can be used in prognostic diagnosis and assessment of the therapeutic efficiency.

Analysis of the chromosome areas nuclear distribution and location of some areas of lymphocyte chromatin in peripheral blood was carried out in 75 patients with verified multiple sclerosis. The control group included 20 healthy volunteers.

Investigation was performed in the real time using apparatus-software complex "Bioni" (Moscow) for clinical and laboratory diagnostics. Software packet enables receiving phase portrait of the cell nucleus and its fragments, file editing and inversion, files subtraction, fluctuation mapping, etc.

It is known, that chromatin of the interphase cellular nucleus has multilevel hierarchic structure. In this case, its structure, location, and functions are closely interrelated. Chromatin being a method to pack DNA, is also a bearer of epigenetic information, i.e. a regulator of gene expression on the account of the fast transformation of its structure in response to intra- and extracellular signals.

The data obtained, are indicative of the possibility to elaborate innovational diagnostic and therapeutic methods based on epigenetic mechanisms of pathogenesis of the majority of pathological processes.

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10074-42, Session 7

Identification of cellular phenotypes of psychiatric disorders with a multimodal quantitative phase digital holography microscopy technique

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Quantitative phase microscopy (QPM) has recently emerged as a powerful label-free technique in the field of living cell imaging. This technology allows the non-invasive measurement of the cell structure and dynamics with a nanometric axial sensitivity. It uses the phase delay of a light wave transmitted through cells, or quantitative phase signal (QPS), caused by the mismatch between the intracellular and extracellular refractive index. Therefore, QPS contains valuable information about cell content and morphology that can be used to derived various cellular parameters, including cellular surface, morphometry, absolute volume as well as its changes, dry mass, membrane fluctuations at the nanoscale and biomechanical properties, transmembrane water permeability and current. We show how quantitative phase digital holographic microscopy (QP-DHM), thanks to its numerical flexibility facilitating parallelization and automation processes, represents an appealing QPM approach for performing original image-based screening, aiming at discriminating a variety of cellular phenotypes. Furthermore, we also present how multimodal imaging involving different QPM approaches based on DHM (including multiwavelength, tomography, etc.), in combination with fluorescence microscopy, represents a powerful method for gaining insight into the biological processes underpinning the observed cellular phenotypes. Practically, as far as cells collected from patients are concerned, the systematic exploration of the different cellular phenotypes represents a highly promising approach to identify new cellular biomarkers of diseases. We have focused on psychiatric disorders by performing QP-DHM multimodal studies of somatic cells (fibroblasts) obtained from patients (skin biopsy) suffering from bipolar disorder and schizophrenia.

10074-43, Session 7

Quantitative phase imaging of retinal cells

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Vision process is ruled by several cells layers of the retina. Before reaching the photoreceptors, light entering the eye has to pass through a few hundreds of micrometers thick layer of ganglion and neurons cells. Macular degeneration is a non-curable disease of themacula occurring with age. This disease can be diagnosed at an early stage by imaging neuronal cells in the retina and observing their death chronically. These cells are phase objects locatedon a background that presents an absorption pattern and so difficult to see with standard imagingtechniques in vivo. Phase imaging

methods usually need the illumination system to be on the opposite side of the sample with respect to theimaging system. This is a constraintand a challenge for phase imaging in-vivo. Recently, the possibility of performing phase contrast imaging from one side using properties of scattering media has been shown. This phase contrast imaging is based on the back illumination generated by the sample itself.

Here, we present a reflection phase imaging technique based on oblique back-illumination. The oblique back-illumination creates a dark field image of the sample. Generating asymmetric oblique illumination allows obtaining differential phase contrast image, which in turn can be processed to recover a quantitative phase image. In the case of the eye, a transcleral illumination can generate oblique incident light on the retina and the choroidal layer.The back reflected light is then collected by the eye lens to produce dark field image.

We show experimental results of retinal phase imagesin ex vivo samples of human and pig retina.

10074-51, Session 7

Measuring optical properties of brain tissues with Alzheimer's disease with quantitative phase imaging

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Imaging brain tissues is an essential part of neuroscience because understanding brain structure provides relevant information about brain functions and alterations associated with diseases. Magnetic resonance imaging and positron emission tomography exemplify conventional brain imaging tools, but these techniques suffer from low spatial resolution around 100 ?m. As a complementary method, histopathology has been utilized with the development of optical microscopy. The traditional method provides the structural information about biological tissues to cellular scales, but relies on labor-intensive staining procedures. With the advances of illumination sources, label-free imaging techniques based on nonlinear interactions, such as multiphoton excitations and Raman scattering, have been applied to molecule-specific histopathology. Nevertheless, these techniques provide limited qualitative information and require a pulsed laser, which is difficult to use for pathologists with no laser training.

Here, we present a label-free optical imaging of mouse brain tissues for addressing structural alteration in Alzheimer's disease. To achieve the mesoscopic, unlabeled tissue images with high contrast and sub-micrometer lateral resolution, we employed holographic microscopy and an automated scanning platform. From the acquired hologram of the brain tissues, we could retrieve scattering coefficients and anisotropies according to the modified scattering-phase theorem. This label-free imaging technique enabled direct access to structural information throughout the tissues with a sub-micrometer lateral resolution and presented a unique means to investigate the structural changes in the optical properties of biological tissues.

10074-52, Session 7

Studying iron-deficiency anemia using simultaneous phase and amplitude microscopy (SPAM)

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Iron deficiency anemia is very prevalent with an incidence of approximately 2 billion cases worldwide, and clinically manifests itself with increased oxidative stress and ineffective erythropoiesis. During erythroid differentiation, heme-regulated eIF2a kinase (HRI) is necessary to coordinate translation of aand b- globin mRNAs with the availability of heme for the production of large amounts of hemoglobin in red blood cells (RBCs). HRI is additionally required for the adaptation to oxidative stress and for reducing ineffective erythropoiesis. In HRI deficiency, excess globins synthesized precipitate and cause proteotoxicity. The molecular basis of erythroid cell adaptation to oxidative stress is not fully understood. In all these pathophysiological stages, RBCs go through pronounced change in their biophysical properties such as morphology and deformability. Understanding the biomechanical and morphological changes red cells go through in all these pathophysiological stages can provide great insight into the disease mechanism complementary to genomic and biological studies. In this study, we are investigating the effect of iron efficiency and various gene knockouts, e.g. HRI, in the red cells of transgenic mice models, using a novel platform technology. Our technique uses complex field measured from interferometric measurements in a new theoretical framework along with subtle changes to the optical properties of the suspension medium.

10074-44, Session 8

Solving the inverse scattering problem in dynamic speckle-field phase microscopy

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Most of the quantitative phase microscopy systems are unable to provide depth-resolved information for measuring complex biological structures. Optical diffraction tomography provides a non-trivial solution to it by 3D reconstructing the object with multiple measurements through different ways of realization. Previously, our lab developed a reflection-mode dynamic speckle-field phase microscopy (DSPM) technique, which can be used to perform depth resolved measurements in a single shot. Thus, this system is suitable for measuring dynamics in a layer of interest in the sample. DSPM can be also used for tomographic imaging, which promises to solve the long-existing "missing cone" problem in 3D imaging. However, the 3D imaging theory for this type of system has not been developed in the literature. Recently, we have developed an inverse scattering model to rigorously describe the imaging physics in DSPM. Our model is based on the diffraction tomography theory and the speckle statistics. Using our model, we first precisely calculated the defocus response and the depth resolution in our system. Then, we further calculated the 3D coherence transfer function to link the 3D object structural information with the axially scanned imaging data. From this transfer function, we found that in the reflection mode excellent sectioning effect exists in the low lateral spatial frequency region, thus allowing us to solve the "missing cone" problem. Currently, we are working on using this coherence transfer function to reconstruct layered structures and complex cells.

10074-45, Session 8

Reconstruction method for extended depth-of-focus limited-angle tomography

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Limited-angle holographic tomography (LAT) is a powerful tool for measuring 3D refractive index distribution in biological microsamples from Petri dishes. However, during the measurement, biological cells introduce

significant diffraction effects into the object laser beam. When the sample is thin, the influence of the diffraction can be limited by conjugating the camera plane with the center of the investigated sample. However, this approach is not sufficient when thicker objects are measured. In this case, one popular solution is to numerically propagate the registered hologram to other planes of the biological sample. This approach has two limitations: (1) propagation errors increase together with the propagation distance, and so this method can be used with thin samples only, (2) it is computationally intensive. Here we propose a hardware-based solution which allows to change a conjugate plane position through the introduction of a liquid tunable lens in LAT system. For each illumination angle, a series of projections with different focal plane positions are recorded, and thus diffraction errors in the neighborhood of these planes are minimized in the final reconstruction. In this paper we describe a modification of the recently proposed Generalized Total Variation Iterative Constraint algorithm. The modification allows to process the data from LAT with tunable lens by independently reconstructing sections of the measurement volume, where each section is associated with one focal plane position. In the final step all sections are stitched to create a reconstruction of the investigated sample. Also, we present an experimental verification of the modified algorithm.

10074-46, Session 8

Optical Projection Tomography via Phase Retrieval Algorithms for Hidden Three Dimensional Imaging

Daniele Ancora, Diego Di Battista, Georgia Giasafaki, Foundation for Research and Technology-Hellas (Greece) and Univ. of Crete (Greece); Stylianos Psycharakis, Evangelos Liapis, Athanasios Zacharopoulos, Giannis Zacharakis, Foundation for Research and Technology-Hellas (Greece)

Optical tomography in biomedical imaging is a highly dynamic field in which non-invasive optical and computational techniques are combined to obtain a three dimensional representation of the specimen we are interested to image. Although at optical wavelengths the phenomena of scattering is the main obstacle to reach diffraction limited resolution, recently several studies have shown the possibility to image even objects fully hidden behind a turbid layer [Nature 491 (7423), 232-234 (2012) and Nature Photonics 8 (10), 784-790 (2014)] exploiting the information contained in the speckle autocorrelation via an iterative phase retrieval algorithm [IEEE Signal Processing Magazine 32 (3), 87-109 (2015)]. In this work we explore the possibility of blind three dimensional reconstruction approach based on the Optical Projection Tomography principles, a widely used tool to image almost transparent model organism such as C. Elegans and D. Rerio. By using autocorrelation information rather than projections at each angle we prove, both numerically and experimentally, the possibility to outperform exact three dimensional reconstructions via a specifically designed phase retrieval algorithm, extending the capability of the projection-based tomographic methods to image behind scattering curtains. The reconstruction scheme we propose is simple to implement, does not require post-process data alignment and moreover can be trivially implemented in parallel to fully exploit the computing power offered by modern GPUs, further reducing the need for costly computational resources.

10074-47, Session 8

Modeling light propagation through scattering medium via numerical solutions of Maxwell's equations *(Invited Paper)*

Snow H. Tseng, Chih-Yao Yang, National Taiwan Univ. (Taiwan)

We employ the pseudospectral time-domain (PSTD) algorithm to model light propagation through a macroscopic scattering medium. We show that with specific amplitude and phase, light can propagate through scattering media and focus. We model explore the feasibility to propagate light the scattering medium with imprecise amplitude or phase. Based upon the numerical experiment, we analyze the degradation due to such imprecision.

10074-48, Session 8

Fundamental and algorithmic sensitivity of phase shifting interferometry

Yizheng Zhu, Chengshuai Li, Virginia Polytechnic Institute and State Univ. (United States)

As quantitative phase imaging (QPI) continues its rapid growth and its functionality and performance continues to improve, understanding its performance from a theoretical point of view becomes important. Phase sensitivity, as a key performance metric for QPI systems, can be dictated by many factors. Some are experimental, such as mechanical stability and component repeatability. These sources of error, in theory and often practically, can be minimized to below detectable level using improved hardware. Some factors, such as shot noise, are however inevitable, thus posing a fundamental limit of sensitivity.

Here we present the noise-limited phase sensitivity using phase shifting interferometry (PSI) as example. We also derive analytical formulas for the sensitivity of popular PSI signal processing algorithms. These expressions represent the performance under the assumption that system experimental deficiencies, such as mechanical vibration and component repeatability, are negligible. Fundamental limit and algorithmic sensitivity of PSI can thus be calculated using only measured data. The formulas provide important insights into fundamental constraints in PSI performance and can be used to guide system design and optimization. Importantly, they show that current algorithms, such as the 4-bucket algorithm, do not necessarily achieve the fundamental limit. We will present the derivations and discuss the implications of these results.

10074-49, Session 8

A fast Fourier ptychographic microscope method with biomedical application

Chaijie Duan, Yawei Kuang, Hui Ma, Graduate School at Shenzhen, Tsinghua Univ. (China)

Fourier ptychographic microscopy (FPM) is a newly reported techniques that breaks the SBP limit, which gets high-resolution (HR) images with large FOV. FPM uses an LED matrix as the illuminating source of the microscope. Each lighted LED corresponds to a low-resolution (LR) image. An HR image is generated from a set of LR images by FPM. Larger illuminating angle provides higher frequency information for the HR image, and vice versa. Therefore, the FPM increases the NA of the low-NA objective lens while maintaining the large FOV. However, since a series of LEDs are used to acquire LR images, the acquisition time is much longer than taking a single image by the traditional microscope. Especially, the dark field images which correspond to the high frequency region in the Fourier space, are more time-consuming for gaining enough signal-to-noise ratio (SNR). This problem limit the application of the FPM especially for dynamic sample imaging. In this paper, we proposed and discussed methods for accelerating FPM acquisition speed, including reducing LR image number, changing illumination configuration and reconstruction algorithm. The methods were tested by using both synthetic data and real biomedical data. The results prove that the new method gave similar results (intensity and phase) to the original FPM method, while using almost only one half of the input image number, and the acquisition time was reduced as well.

Conference 10075: Biophysics, Biology and Biophotonics II: the Crossroads

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10075-1, Session 1

Coherence switching of a vertical-cavity semiconductor-laser for multimode biomedical imaging *(Invited Paper)*

Hui Cao, Sebastian Knitter, Changgeng Liu, Brandon Redding, Mustafa Kezar Khokha, Michael Andrew Choma, Yale Univ. (United States)

Speckle formation is a limiting factor when using coherent sources for imaging and sensing, but can provide useful information about the motion of an object. Illumination sources with tunable spatial coherence are therefore desirable as they can offer both speckled and speckle-free images. Efficient methods of coherence switching have been achieved with a solid-state degenerate laser, and here we demonstrate a semiconductor-based degenerate laser system that can be switched between a large number of mutually incoherent spatial modes and few-mode operation.

Our system is designed around a semiconductor gain element, and overcomes barriers presented by previous low spatial coherence lasers. The gain medium is an electrically-pumped vertical external cavity surface emitting laser (VECSEL) with a large active area. The use of a degenerate external cavity enables either distributing the laser emission over a large (~1000) number of mutually incoherent spatial modes or concentrating emission to few modes by using a pinhole in the Fourier plane of the self-imaging cavity. To demonstrate the unique potential of spatial coherence switching for multimodal biomedical imaging, we use both low and high spatial coherence light generated by our VECSEL-based degenerate laser for imaging embryo heart function in Xenopus, an important animal model of heart disease. The low-coherence illumination is used for high-speed (100 frames per second) speckle-free imaging of dynamic heart structure, while the high-coherence emission is used for laser speckle contrast imaging of the blood flow.

10075-2, Session 1

Lasers inside live cells

Matja? Humar, Jo?ef Stefan Institute (Slovenia) and Univ. of Ljubljana (Slovenia) and Harvard Medical School, Massachusetts General Hospital (United States); Seok-Hyun Yun, Harvard Medical School, Massachusetts General Hospital (United States) and Harvard-MIT Health Sciences and Technology (United States) and Jo?ef Stefan Institute (Slovenia)

We have demonstrated a laser completely embedded within a single live cell. The lasers were made out of solid fluorescent beads few microns in diameter. These laser beads were fed to live cells in culture, which engulfed the lasers within a few hours. The lasers can act as very sensitive sensors, enabling us to better understand cellular processes. For example, we measured small changes in the refractive index of the cytosol, which is related to the concentration of chemical constituents within the cells. Further, lasers were used for cell tagging. Each laser within a cell emits light with a slightly different fingerprint that can be easily detected and used as a barcode to tag the cell. With careful laser design and multiplexing, up to a trillion cells (1,000,000,000,000) could be uniquely tagged. This would enable to uniquely tag every single cell in the human body, providing the ability the study cell migration including cancer metastasis. Further, by using a micro pipette droplets of high refractive index oil containing fluorescent dye were injected into a cell. By analyzing the light emitted by a droplet laser, we can measure that deformation and calculate the forces acting within a cell. Finally, we realized that fat cells already contain lipid droplets, which can work as natural lasers. The operation of fat cell lasers was demonstrated in the skin tissue.

10075-3, Session 1

Monitoring adsorption of gold nanoparticles on gold nanodisk array using dark-field hyperspectral microscopy

Fusheng Zhao, Oussama Zenasni, Jingting Li, Wei-Chuan Shih, Univ. of Houston (United States)

Localized surface plasmon resonance (LSPR) arises from the interaction of light with noble metal nanoparticles, which induces a collective oscillation in the free electrons. The size and shape of the metallic nanostructure significantly impact LSPR frequency and strength. Nanoplasmonic sensor has become a recent research focus due to its significant signal enhancement and robust signal transduction measured by extinction spectroscopy, fluorescence, Raman scattering, and absorption spectroscopy. Dark-field microscopy, in contrast, reports the scattered photons after light-matter interactions. In this case, the nanoparticles can be understood as dipole radiators whose free electrons oscillate in concert. Coupled with spectroscopy, this platform allows the collection of plasmonically scattered spectra from gold nanoparticles.

Plasmonic coupling between electron-beam lithography patterned gold nanodisks (AuND) and colloidal gold nanoparticles (AuNP) can change the plasmonic resonance of the original entities, and can be effectively studied by dark-field hyperspectral microscopy. Typically, a pronounced redshift can be observed when plasmonic coupling occurs. When these nano-entities are functionalized with interactive surface moieties, biochemistry and molecular processes can be studied. In this paper, we will present the capability of assessing the process of immobilizing streptavidin-functionalized AuNPs on an array of biotin-terminated AuNDs. By monitoring changes in the LSPR band of AuNDs, we are able to evaluate similar processes in other molecular systems.

In addition, plasmon coupling induced scattering intensity variations can be measured by an electron-multiplied charge-coupled device camera for rapid in situ monitoring. This method can potentially be useful in studying dynamic biophysical and biochemical processes in situ.

10075-4, Session 1

Single-molecule DNA hybridization on nanoporous gold nanoparticle array chip

Jingting Li, Fusheng Zhao, Wei-Chuan Shih, Univ. of Houston (United States)

DNA hybridization, where two single-stranded DNA (ssDNA) molecules form duplex through non-covalent, sequence-specific interactions, is a fundamental process in biology. Developing a better understanding of the kinetics and dynamic aspects of hybridization will help reveal molecular mechanisms involved in numerous biomolecular processes. To this end, sequence-specific detection of hybridization at the single-molecule level has been instrumental and gradually become a ubiquitous tool in a wide variety of biological and biomedical applications such as clinical diagnostics, biosensors, and drug development. Label-free and amplification-free schemes are of particular interest because they could potentially provide in situ monitoring of individual hybridization events, which may lead to techniques for discriminating subtle variations due to single-base modification without stringency control or repetitive thermal cycling. To further increase experimental robustness and productivity and reduce complexity, single-step assays are highly desirable.

Nanoporous gold nanoparticle (NPG-NP) array chip showcases tunable pore and ligament sizes ranging from nanometers to microns. The nanoporous structure and sub-wavelength nanoparticle shape contribute to its unique LSPR properties. NPG-NP features large specific surface area and high-density plasmonic field enhancement known as “hot-spots”. Hence,

NPG-NP array chip has found many applications in nanoplasmonic sensor development. In our recent studies, we have shown that NPG-NP array chip can be utilized for high-sensitivity detection by various enhanced spectroscopic modalities, as photothermal agents, and for disease biomarker detection.

In this paper, we discuss results on detecting single-molecule DNA hybridization on functionalizing NPG-NP array chip with unique bio-recognition elements towards both high sensitivity and specificity.

10075-5, Session 1

Multiplexed lasing in tissues

Yu-Cheng Chen, Qiushu Chen, Xudong Fan, Univ. of Michigan (United States)

Lasing within a biological specimen for differentiation of specific targets has been an attractive topic recently. To date, the tissue lasers have been demonstrated using the random lasers with single fluorophores, showing distinct advantages over fluorescence based methods in analyzing subtle structures of tissues at the microscale and sub-microscale. However, random lasers are unable to provide repeatable, comparable, and precise laser signals from multiple targets. In this work, we demonstrated for the first time multiplexed tissue lasing when the tissues doped with different dyes were sandwiched within a high-Q Fabry-Perot cavity. We achieved precise and distinctive lasing signals from FITC in muscular tissues and BODIPY in adipose tissues by pumping at the same excitation wavelength (465 nm). We found that despite the large fluorescence spectral overlap between the two fluorophores, the laser emissions were still distinguishable within a complex environment. We also investigated the lasing threshold for different tissue thicknesses, structures, and dye concentrations. Theoretical analysis was performed to address the phenomenon observed in various tissue geometries. Our study reveals the unique capabilities of tissue lasing over random lasers, including much lower threshold, much lower dye concentrations, higher signal-to-noise ratio, and multiplexed detection at precise positions. The multiplexed tissue lasing can be applied to a variety of tissues and differentiation of various diseases and cancers. The significance of this work is to pioneer a novel approach for future medical diagnostics and screening in tissues, as well as monitoring, identification, and examination of biological transformations in tissue engineering.

10075-30, Session 1

Surface cleaning and functionalization for single molecule fluorescence imaging and spectroscopy

Janel Davis, Biqin Dong, Cheng Sun, Hao F. Zhang, Northwestern Univ. (United States)

The rise of super-resolution fluorescence imaging and single molecule spectroscopy techniques has presented the opportunity to study molecular interactions with nanometer accuracy. In order for these experiments to be successful, it is of the upmost importance to utilize reliable sample preparation techniques. It has been found that contaminants on and possibly in the silica surface significantly affect the quality of data collected from these single molecular studies. Here, we first summarized widely used techniques for silica surface cleaning and biochemical functionalization. After performing each surface cleaning treatment, organic residues and fluorescence centers were examined by excitations at various wavelengths and power. To quantitatively access the surface cleanliness, the number and intensity of fluorescing clusters on the silica substrate was further recorded for statistical analysis. Additionally, spectroscopic analysis was performed on the functionalized surfaces to determine the contribution of surface functionalization to the spectral background. These experiments have allowed us to optimize common surface cleaning and functionalization protocols for single molecule studies by reducing the background signals. Overall, our experiments demonstrate the importance of surface preparation in experiment quality and reliability. Finally, by assessing the reliability and repeatability of the cleaning and functionalization of glass surfaces; stronger, more consistent conclusions can be made from single-molecule studies.

10075-6, Session 2

Real-time imaging of cell traction and growth *(Invited Paper)*

Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Despite the crucial role of mechanochemical signaling in phenomena such as cell migration, proliferation, and differentiation, measuring the cell-generated forces at the interface with the extracellular matrix remains a challenging task. An ideal method would provide continuous, non-destructive images of the force field applied by cells, over broad spatial and temporal scales. Toward this goal, we developed Hilbert phase dynamometry (HPD) as a new approach for real-time monitoring of cell traction forces. HPD relies on extracting the displacement field in a deformable substrate, which is chemically patterned with a fluorescent grid. The displacements introduced by the cell are captured by the phase of the periodic signal associated with the grid and we retrieve the phase information by translating concepts from holography. The displacement field is uniquely converted into forces by solving an elasticity inverse problem. Because the measurement of displacement only uses the epi-fluorescence channel of an inverted microscope, we can simultaneously achieve measurements in transmission. In particular, we performed quantitative phase imaging and extracted cell mass on the same field of view. We studied mesenchymal stem cells and found that cells undergoing osteogenesis and adipogenesis, exerted larger and more dynamic stresses than their precursor. Our results indicate that the MSCs develop the smallest forces and growth rates. We anticipate that simultaneous cell growth and traction measurements will improve our understanding of mechanotransduction, particularly during dynamic processes where the matrix properties provide context to guide cells towards a physiological or pathological outcome, e.g., tissue morphogenesis, or cancer metastasis.

10075-7, Session 2

Nanoscale chromatin structure characterization for optical applications: a transmission electron microscopy study

Yue Li, Lusik Cherkezyan, Di Zhang, Luay Almassalha, Eric Roth, John Chandler, Reiner Bleher, Hariharan Subramanian, Vinayak P. Dravid, Vadim Backman, Northwestern Univ. (United States)

Structural and biological origins of light scattering in cells and tissue are still poorly understood. We demonstrate how this problem might be addressed through the use of transmission electron microscopy (TEM). For biological samples, TEM image intensity is proportional to mass-density, and thus proportional to refractive index (RI). By calculating the autocorrelation function (ACF) of TEM image intensity of a thin-section of cells, we essentially maintain the nanoscale ACF of the 3D cellular RI distribution, given that the RI distribution is statistically isotropic. Using this nanoscale 3D RI ACF, we can simulate light scattering through biological samples, and thus guiding many optical techniques to quantify specific structures. In this work, we chose to use Partial Wave Spectroscopy (PWS) microscopy as a one of the nanoscale-sensitive optical techniques. Hela cells were prepared using standard protocol to preserve nanoscale ultrastructure, and a 50-nm slice was sectioned for TEM imaging at 6 nm resolution. The ACF was calculated for chromatin, and the PWS mean sigma was calculated by summing over the power spectral density in the visible light frequency of a random medium generated to match the ACF. A 1-μm slice adjacent to the 50-nm slice was sectioned for PWS measurement to guarantee identical

chromatin structure. For 33 cells, we compared the calculated PWS mean sigma from TEM and the value measured directly, and obtained a strong correlation of 0.69. This example indicates the great potential of using TEM measured RI distribution to better understand the quantification of cellular nanostructure by optical methods.

10075-8, Session 2

Optical properties of melanosomes in retinal pigmented epithelium

Ji Yi, Lei Zhang, Boston Univ. (United States)

Melanosome is an organelle for synthesis, storage and transport the melanin, a major intrinsic pigment. In retinal pigmented epithelium (RPE), it is generally accepted that melanosome plays a critical photoprotective role, and it has been shown that that loss of melanin from RPE could be an early event towards age-related macular degeneration (AMD). Meanwhile, melanosome is also the major contributor to the optical properties of RPE, due to its high refractive index and the strong optical absorption of melanin. Therefore, a characterization and understanding the optical properties of melanin is of great interest to relate the physical and chemical changes of melanosomes, and their fundamental roles in RPE-related retinal diseases such as AMD. Here, we present a theoretical study to characterize the full optical properties of melanosomes. We modeled melanosomes as uniformly melanin filled spheroids, based on their morphology under transmission electron microscopy. T-matrix method was used to simulate the wavelength dependent total scattering, backscattering, absorption cross sections, and anisotropy factor. We verified our simulation on backscattering cross section of melanosome by comparing optical coherence tomography taken in visible and NIR ranges. In addition, we studied the changes of the optical properties of melanosomes on melanin bleaching. The results suggested a spectroscopic mechanism for optical detection of melanin loss by inverse spectroscopic optical coherence tomography.

10075-9, Session 2

Could low level laser therapy and highly active antiretroviral therapy lead to complete eradication of HIV-1 in vitro?

Masixole Yvonne Lugongolo, Sello Lebohang Manoto, Saturnin Ombinda-Lemboumba, Council for Scientific and Industrial Research (South Africa); Malik Maaza, Univ. of South Africa (South Africa) and iThemba LABS-National Research Foundation (South Africa); Patience T. Mthunzi-Kufa, Council for Scientific and Industrial Research (South Africa) and Univ. of South Africa (South Africa)

Human immunodeficiency virus (HIV-1) infection remains a major health problem despite the use of highly active antiretroviral therapy (HAART), which has greatly reduced mortality rates. Due to the unavailability of an effective vaccine or a treatment that would completely eradicate the virus, the quest for new and combination therapies continues. In this study we explored the influence of Low Level Laser Therapy (LLLT) in HIV-1 infected and uninfected cells. Literature reports LLLT as widely used to treat different medical conditions such as diabetic wounds, sports injuries and others. The technique involves exposure of cells or tissue to low levels of red and near infrared laser light. Both HIV infected and uninfected cells were laser irradiated at a wavelength of 640 nm with fluencies ranging from 0 to 10 J/cm2 and cellular responses were assessed 24 hours post laser treatment. In our studies, laser therapy had no inhibitory effects in HIV-1 uninfected cells as was indicated by the cell morphology and proliferation results. However, laser irradiation enhanced cell apoptosis in HIV-1 infected cells as the laser fluencies increased. This led to further studies in which laser irradiation would be conducted in the presence of HAART to determine whether HAART would minimise the detrimental effects of laser irradiation in infected cells.

10075-10, Session 3

Convergence of nano-imaging, physics and biology: from understanding cancer biology to early detection and therapeutics *(Invited Paper)*

Vadim Backman, Northwestern Univ. (United States)

Initiation of carcinogenesis is accompanied by alterations in tumor microenvironment, cellular metabolism and epigenetics. Understanding these early events depends on our ability to image these subtle nanoarchitectural and functional processes. The talk discusses a suite of novel in vivo and in vitro optical imaging techniques to quantify intracellular and extracellular morphology and physiology. The talk also discusses how optical nano-imaging, the modeling of complex molecular interactions involved in gene expression from the physics perspective, and a systems approach to biology can lead to new fundamental insights into the origins of cancer, a platform for highly accurate, cost-effective and non-invasive cancer screening, and new highly effective physico-chemical approaches to anti-cancer therapy.

10075-11, Session 3

Interferometric imaging *(Invited Paper)*

Poorya Hosseini, Renjie Zhou, Zahid Yaqoob, Peter T. C. So, Massachusetts Institute of Technology (United States)

From sickle cell anemia to cancer metastasis and genetic disorders such as progeria and muscular dystrophies, there are major structural and functional changes at the molecular, cellular and organ level. Interferometric imaging has resulted in optical mass sensors for cells with femtogram sensitivity enabling unprecedented precision in studying cell cycle control and optical rheometer discovering new physical markers for evaluating pharmaceutical treatments for blood diseases. We will discuss several recent advances for probing biomechanics of cellular nucleus, for high throughput sorting of messchychemal stemm cells, and for classifying individual sickle red blood cells with molecular specificity.

10075-12, Session 3

The nanoscopic topology of chromatin: implications of physical structure and molecular function

Luay Almassalha, Biqin Dong, Yolanda Stypula-Cyrus, Ben E. Urban Jr., John E. Chandler, The-Quyen Nguyen, Cheng Sun, Igal Szleifer, Hao F. Zhang, Vadim Backman, Northwestern Univ. (United States)

For decades, advances in molecular biology have increased our understanding of the regulatory elements of chromatin, the structure housing most eukaryotic genetic information. While this has provided tremendous insights into the role of chromatin on cellular behavior, these techniques cannot provide information on the spatio-temporal organization of the genome. Recent evidence has suggested that the three dimensional organization of chromatin and its temporal evolution play a critical role in process such as transcription, DNA repair, and replication. Only recently have there been the development of integrated approaches to visualize the nanoscale intracellular structures formed by nucleic acids, such as chromatin, in non-perturbed, structurally and dynamically complex cellular systems. In this work, we discuss the implications of the recently observed fractal topology of chromatin spanning the length-scales as small as 20nm and into the micron scale. Using the newly discovered capability to directly visualize DNA without extrinsic contrast, we perform a quantitative analysis of the deeply sub-diffractional (20-60nm) organization of chromatin.

Extending these findings into observations in live cells using other optical methods, microarray gene analysis, and theoretical modeling, we discuss the role of the physical structure of chromatin on transcriptional regulation.

10075-13, Session 3

FDTD based model of ISOCT imaging for validation of nanoscale sensitivity

Aya Eid, Di Zhang, Northwestern Univ. (United States); Ji Yi, Boston Univ. (United States); Vadim Backman, Northwestern Univ. (United States)

Many of the earliest structural changes associated with neoplasia occur on the micro and nanometer scale, and thus appear histologically normal. Our group has established Inverse Spectroscopic OCT (ISOCT), a spectral based technique to extract nanoscale sensitive metrics derived from the OCT signal. Thus, there is a need to model light transport through relatively large volumes (> 50 um^3) of media with nanoscale level resolution.

Finite Difference Time Domain (FDTD) is an iterative approach which directly solves Maxwell's equations to robustly estimate the electric and magnetic fields propagating through a sample. The sample's refractive index for every spatial voxel and wavelength are specified upon a grid with voxel sizes on the order of ?/20, making it an ideal modelling technique for nanoscale structure analysis.

Here, we utilize the FDTD technique to validate the nanoscale sensing ability of ISOCT. The use of FDTD for OCT modelling requires three components: calculating the source beam as it propagates through the optical system, computing the sample's scattered field using FDTD, and finally propagating the scattered field back through the optical system. The principles of Fourier optics are employed to focus this interference field through a 4f optical system and onto the detector.

Three-dimensional numerical samples are generated from a given refractive index correlation function with known parameters, and subsequent OCT images and mass density correlation function metrics are computed. We show that while the resolvability of the OCT image remains diffraction limited, spectral analysis allows nanoscale sensitive metrics to be extracted.

10075-14, Session 4

Optical screens of primary organoids for personalized cancer therapy *(Invited Paper)*

Melissa C. Skala, Morgridge Institute for Research (United States) and Univ. of Wisconsin-Madison (United States); Alexandra J. Walsh, National Research Council (United States); Rebecca S. Cook, Vanderbilt Univ. (United States)

Primary tumor organoids are a robust three-dimensional model of human cancer. Hundreds to thousands of organoids can be grown from a single tissue biopsy, which provides replicates of a patient-specific tumor for drug testing. Recent studies have shown that drug response in organoids correlates with host drug response. Non-invasive optical imaging techniques provide dynamic information on organoid function to rapidly assess organoid behavior and drug response at sub-cellular resolution. Therefore, functional optical imaging of organoids could enable accurate, high-throughput screens of drug response for individualized cancer treatment.

10075-15, Session 4

Label-free imaging of chromatin nanoscale dynamics: integrated imaging of the cellular nanoarchitecture and molecular motion

Scott Gladstein, Luay Matthew Almassalha, Lusik Cherkezyan, John E. Chandler, Andrew Stephens, Di Zhang, Wenli Wu, Hariharan Subramanian, John Marko, Igal Szleifer, Vadim Backman, Northwestern Univ. (United States)

The structure of chromatin is a complex organization consisting of DNA, histones, and many other conjugated macromolecules. This topology has been studied using many techniques in an attempt to understand important cellular processes such as gene transcription, replication, DNA repair, and apoptosis. Previously, our lab has developed Live Cell Partial Wave Spectroscopic (PWS) microscopy for label-free nanoscale (20-200nm) imaging of the chromatin physical topology. While studying the role of chromatin structure on these molecular processes can provide a lot of insights on biological function, all of these processes are dependent, not just on the static structure of chromatin, but the movement of molecules such as DNA and nuclear proteins (e.g., RNA polymerases, nucleases, helicases, etc.). In this work, we considerably expand on the capabilities of Live Cell PWS microscopy by employing temporal interference to provide information about molecular motion in live cells. Specifically, we utilize both spectral and temporal interference to study the structure-function relationship between cellular nanoarchitecture and intranuclear motion. Employing these modalities, we investigate potential structural regulators of intranuclear motion such as the actin cytoskeleton, nuclear lamina, and chromatin itself. Finally, we study changes in chromatin structure and intranuclear dynamics in response to UV induced DNA damage. In total, we demonstrate that regulation of the structure and motion of chromatin depends on the actin cytoskeleton, the nuclear lamina, and the organization of chromatin itself.

10075-16, Session 4

Modeling cell-matrix interaction in ovarian cancer through image based 3D biomimetic scaffolds created by multiphoton excited fabrication *(Invited Paper)*

Visar Ajeti, Manish Patankar, Kevin W. Eliceiri, Paul J. Campagnola, Univ. of Wisconsin-Madison (United States)

A profound remodeling of the extracellular matrix (ECM) occurs in human ovarian cancer but it unknown how this affects tumor growth, where this understanding could lead to better diagnostics and therapeutic approaches. Here, we investigate the role of these ECM alterations by using multiphoton excited (MPE) polymerization to fabricate biomimetic models of the ovarian stroma to investigate operative cell-matrix interactions in invasion/metastasis. This process is akin to 3D printing except is performed at much higher resolution and with the proteins that comprise the native ECM. We use this technique to create collagen scaffolds with complex, 3D submicron morphology, where the scaffold designs are derived directly from Second Harmonic Generation (SHG) images of normal, high risk, benign tumors, and malignant human ovarian tissues. The models are seeded with different cancer cell lines and this allows decoupling of the roles of cell characteristics (metastatic potential) and ECM structure and composition (normal vs cancer) on adhesion/migration/proliferation dynamics. Through timelapse imaging and immunostaining, we found the malignant stromal structure promoted enhanced migration persistence and cell proliferation and also cytoskeletal alignment. Moreover, the method allows varying fiber properties such as diameters and characteristic frequency as well as overall alignment. While collagen alignment is known to affect cell dynamics, we further found

that the migration dynamics are highly dependent upon the morphological properties of the fibers themselves. These models cannot be synthesized by other conventional fabrication methods and we suggest the MPE image-based fabrication method will enable a variety of studies in cancer biology.

10075-18, Session 4

Label-free in vivo imaging of the tumor microenvironment in zebrafish

Marco Andreana, Medizinische Univ. Wien (Austria); Martin Distel, Children's Cancer Research Institute, St. Anna Kinderkrebsforschung e.V. (Austria); Matthias Salas, Aart J. Verhoef, Alma del Carmen Fernandez Gonzalez, Wolfgang Drexler, Angelika Unterhuber, Medizinische Univ. Wien (Austria)

Zebrafish have emerged as an attractive animal system for the generation of zebrafish cancer models relevant to human diseases. The tumors developed in various organ sites are histologically and genetically similar to human malignancies and the zebrafish possess the entire major immune cell lineages found in humans. Since hematopoietic gene functions are conserved in zebrafish and vice versa, it can be suggested that findings in zebrafish can be directly translated to humans. In addition, zebrafish is a cost-effective model organism and tumor initiation stages in larval zebrafish embryos are accessible to fluorescent microscopy but requires staining. We report a newly developed optical imaging platform as powerful tool for longitudinal in vivo studies of the tumor microenvironment in zebrafish in transmission and reflection mode. Our simple and cost-effective label-free multimodal imaging system fuses hyperspectral CARS, SHG and TPEF with OCT to facilitate label-free extraction of functional, molecular and morphological information by high-speed, three-dimensional wide-field screening of the zebrafish and high-resolution zoom-in capabilities. Lipids play important and diverse roles in cells and the lipid content is significantly altered with tumor progression. We investigate these changes with our lipid CARS channel. Furthermore, the molecular organization, amount and distribution of fibrillary collagen are important for the structural and mechanical properties of tissue thus playing an important role in cancer. SHG reveals changes in the orientation of collagen with tumor progression in a second channel. Autofluorescencing molecules can be detected with TPEF in a third channel. All channels are acquired simultaneously and coregistered with wield-field OCT.

10075-19, Session 5

Imaging abnormalities in cancer with advanced microscopy tools *(Invited Paper)*

Conor L. Evans, Wellman Ctr. for Photomedicine (United States)

Cancer is marked by major abnormalities in growth, metabolism, perfusion, and a host of related macro- and micro-environmental alterations. These factors are heterogeneous both within and between tumors, giving rise to a largely unmapped landscape of tumor growth and treatment response. We are developing a set of advanced imaging tools and probes that can be used to map and understand the impact of these spatiotemporal heterogeneities.

10075-20, Session 5

Serogroup-specific interactions of lipopolysaccharide with supported lipid bilayer assemblies

Heather M. Mendez, The Univ. of New Mexico (United States); Loreen R. Stromberg, Iowa State Univ. of Science and Technology (United States); Steven W. Graves, The Univ. of New Mexico (United States); Rodney A. Moxley, Univ. of Nebraska-Lincoln (United States); Gabriel Montaño, Ctr. for Integrated Nanotechnology, Los Alamos National Lab. (United States); Harshini Mukundan, Los Alamos National Lab. (United States)

Amphiphilic moieties such as lipoglycans and glycoproteins play an important role in many host-pathogen processes, and understanding them is critical to developing better solutions to target infectious diseases. Lipopolysaccharides (LPS) are one such category of amphiphilic lipoglycans. LPS is comprised of a hydrophobic lipophilic domain that partitions into the outer membrane of Gram-negative bacteria. Biophysical interaction of LPS with lipid bilayers remains unclear, due in part to not taking into consideration the amphiphilic biochemistry of LPS. The goal of this work was to incorporate fluorescence microscopy and atomic force microscopy analysis to study the interaction between LPS and supported lipid bilayer assemblies. (SLBAs). LPS was extracted from six representative serogroups of Shiga-toxin producing Escherichia coli and used to investigate the interaction of LPS serogroups with SLBAs, while varying environmental conditions, such as LPS concentration, ionic strength, and temperature. Incorporation of cholesterol and membrane proteins were also introduced to SLBAs to understand LPS-membrane interactions with complex membranes. In addition, pathophysiological potential amongst LPS serogroups stem from chemical structural variabilities within the O-ags of LPS. To understand how chemical structural variability of LPS between serogroups contributes to variability in behavior of biomimetic systems, structural analysis of the LPS and O-antigens were performed via one and two dimensional 1H and 13C nuclear magnetic resonance spectroscopy. Future work will utilize an in vitro cell system to mimic LPS-membrane interactions seen in our model membrane systems.

10075-21, Session 5

Investigation of HIV-1 infected and uninfected cells using the optical trapping technique

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Optical trapping has emerged as an essential tool for manipulating single biological material and performing sophisticated spectroscopy analysis on individual cell. The optical trapping technique has been used to grab and immobilize cells from a tightly focused laser beam emitted through a high numerical aperture objective lens. Coupling optical trapping with other technologies is possible and allows stable sample trapping, while also facilitating molecular, chemical and spectroscopic analysis. For this reason, we are exploring laser trapping combined with laser spectroscopy as a potential non-invasive method of interrogating individual cells with a high degree of specificity in terms of information generated. Thus, for the delivery of as much pathological information as possible, we use a home build optical trapping and spectroscopy system for probing human immunodeficiency virus (HIV-1) infected and uninfected single cells. Briefly, our experimental rig comprises an infrared continuous wave laser at 1064 nm with power output of 1.5 W, a 100X high numerical aperture objective used to capture and immobilise individual cell samples as well as an excitation source. Spectroscopy spectral patterns obtained by the 1064 nm

laser beam excitation provide information on HIV-1 infected and uninfected cells. We present these findings which may be valuable for the development of an HIV-1 point of care detection system.

10075-22, Session 5

Molecular skin model for pharmacological studies and cosmetology

Erika T. Sato, Herculano S. da Silva Martinho, Univ. Federal do ABC (Brazil)

The water permeability study of the different skin layers as well as the permeability of the stratum corneum drugs are determinant factors to obtain a topical pharmaceutical formulation. Several tests are performed using artificial and natural skins, especially pork's skin, to pharmaco be approved for human use. Besides the ethical implications, these tests permeate months to obtain a single viable formulation. Although the use of computational models present growth in the cosmetology and pharmacology areas, a computational quantum model to describe skin changes and adduce drug ways into skin is not known yet. Raman spectroscopy describes well the water presence in biosystems and it has good description for quantum computer models. Thus, it was created a DFT (Density Functional Theory) model able to optimize the drugs formulation and understand the process of permeability in the different tissue layers using molecular dynamics. The model starting from the skin model already developed by Sato's methodology (Sato et al. [1]). For this, interesting drugs were added to the model, trying to analyze their permeabilities and chemical structural changes, comparing each computational results with the Franz cells, in order to validate the model and testing other components. They will also be observed the characteristic peaks of the samples involved as a way to also check the feasibility of the model. From there, other physical, chemical and biological properties can be calculated.

[1] SATO, E. T. et al., Molecular model for hydrated biological tissues. Physical Review. E, v. 91, p. 063310, 2015

10075-24, Session PSun

Photophysical and photochemical changes in lysozyme induced by UV light

Odete Sofia Lopes Goncalves, Aalborg Univ. (Denmark); Addison Marshall, Jean-Sebastien Bouillard, Ali M. Adawi, The Univ. of Hull (United Kingdom); Maria Teresa Neves-Petersen, Steffen B. Petersen, Aalborg Univ. (Denmark)

Light can induce changes in proteins in various ways. Photo-oxidation is one of the processes that can be induced by UVB light leading to photoproducts formation or electron ejection from aromatic residues sidechains, causing structural changes. Conformational changes in proteins can have an important role in protein function. By means of fluorescence spectroscopy it is possible to detect these changes since proteins display intrinsic fluorescence due to the presence of aromatic residues such as tryptophan (Trp) and tyrosine (Tyr). These aromatic residues can act as molecular probes enabling the detection of multiple events.

Hen egg white lysozyme is a commonly used and well-studied model protein with a well-known structure. It is a 129 amino acid long monomeric catalytic enzyme displaying 4 disulfide bridges, 6 Trp and 3 Tyr residues. We have investigated the 280nm induced photochemical and photophysical changes in lysozyme at pH 7.4 in PBS using both Fluorescence spectroscopy and Raman spectroscopy. We observe a progressive decay of the Trp fluorescence emission signal at 330 nm as a function of illumination time. The decay curve can be fitted to a double exponential decay with time constants 0.0009927 and 0.0002324 and pre-exponentials 2.63*10^6 and 1.346*10^6 respectively. Surface enhanced Raman spectra have been obtained from ~400 cm-1 to 3000 cm-1 at discrete times during the entire illumination period. Marked and distinct changes are observed at several wavelengths.

10075-26, Session PSun

Ab-initio DFT computational model of vibrational spectroscopy data for squamous cell carcinoma

Daiana Ribeiro Bortoletto, Univ. Federal do ABC (Brazil); Cássio A. Lima, Denise Zezell, Univ. de São Paulo (Brazil); Herculano S. da Silva Martinho, Univ. Federal do ABC (Brazil)

The optical biopsy using vibrational spectroscopy is emerging as a technique with good sensitivity and specificity in the diagnosis of diseases. Non-melanoma skin cancers represents for 95% of skin cancers, among which squamous cell carcinoma is the most aggressive form [1]. Considering that early diagnosis is critical to achieving treatment with favorable results, the search for new techniques and methods of diagnosis has become a promising area. For better understand how biochemical changes translate into structural changes are required further study and computer simulation is the appropriate tool. A computational model for the tumor tissue was built from a collagen peptide, with different degrees of hydration and the presence of water contained in its structure, and compare it with experimental carcinogenesis data on skin tissue to the Amide bands, obtained by FTIR. Molecular dynamics calculations in the Amide spectral window were performed based on the Sato's methodology (Sato et al. [2]). We identified the frequencies with variations between normal and tumor tissue for the models that best fit the experimental data [1]. Our analysis indicated the hydrated model with 8 waters furnished the best description of tumor experimental data. The normal data were best described by the model with 4 waters.

[1] LIMA, C. A. et al., ATR-FTIR Spectroscopy for the Assessment of Biochemical Changes in Skin Due to Cutaneous Squamous Cell Carcinoma, Int. J. Mol. Sci. 2015, 16(4), 6621-6630.

[2] SATO, E. T. et al., Molecular model for hydrated biological tissues. Physical Review. E, v. 91, p. 063310, 2015.

10075-27, Session PSun

Oxidatively generated base damage to DNA in aqueous solutions by femtosecond laser-induced low density plasma multi-channels controlled with a spatial light modulator

Hakim Belmouaddine, Guru S. Madugundu, Simon Lefebvre, Richard J. Wagner, Léon Sanche, Daniel Houde, Univ. de Sherbrooke (Canada)

This study addresses a new paradigm in radiobiology, namely the generation of femtosecond laser-induced "cold" low density plasma for the highly localized deposition of energy at sub-cellular scales in systems of biological interest. In such systems, plasma-mediated effects on organic molecules, including DNA, are related to the radiation chemistry of water and involve interactions with radical oxygen species and secondary low energy electrons produced by the plasma.

To better understand the radiation chemistry following from the generation of low density plasma in aqueous environments, we harness the multi-filamentation of powerful near-infrared femtosecond laser pulses to achieve the self –regulated production of spatially homogeneous low density plasma spots in aqueous solutions. An original particularity of this work is the use of a spatial light modulator to control the filamentation process. Rather than the random bundle of entangled low density plasma channels, or filaments, usually produced during the non-linear propagation of the laser beam, the spatial light modulator allows a programmable matrix of mono-filaments to obtain a more ubiquitous and homogeneous energy deposition.

The present method of irradiation has allowed us to perform HPLC-MS

and LC-MS/MS analysis to determine, quantify and compare the yields of 15 oxidation products generated by both the laser irradiation and a conventional source of ionizing radiation (Gamma-Rays) in aqueous solutions of isolated oligonucleotides and DNA. Our analysis shows that each filament behaves as an independent intense micro beam of ionizing radiation capable of yielding complex DNA damage.

10075-28, Session PSun

Rotational and translational diffusion in ficoll solutions

Elton Jhamba, Zakaria M'Rah, Hacene Boukari, Delaware State Univ. (United States); Mohammad A Khan, Delaware State University (United States)

We report fluorescence correlation spectroscopy (FCS) and fluorescence anisotropy (FA) measurements of Alexa488 fluorophores (MW≈570 Da) and FITC-labeled Ficoll (MW≈70 kDa) diffusing in non-fluorescent –hence "invisible"- Ficoll solutions under thermal fluctuations. We determine changes of the apparent rotational and translational diffusion coefficients with systematic increase of Ficoll concentration up to 1200 mg/ml. Notably, the changes cannot be accounted for by the corresponding changes of the bulk viscosity of the Ficoll solutions as would be suggested by the Stokes-Einstein relations for both diffusion coefficients. Instead, we analyze the data with the entropic model proposed by de-Gennes and his collaborators, and fit each set of data with a stretched exponential [exp(-acˆn)] with n being related to the quality of the solvent. The fits of the diffusion data yield n-value close to one, suggesting a theta-like behavior of the host Ficoll-water system. However, the a-value depends on the size of the nanoprobe – the larger the nanoprobe, the larger a-value. Further, the a-value for translation is larger than that of rotation, indicating dissimilar local entropic effects on the rotation and translation.

10075-29, Session PSun

Nature-inspired signal processing

Frank Edughom Ekpar, Imatronics Worldwide (Japan)

This paper establishes the foundational principles and practice for a unified theory of arbitrary information management by disclosing systems, devices and methods for the management of substrates or biological substrates. In this context, a substrate is any aspect of any entity that is capable of responding to or emitting stimuli irrespective of whether the stimuli actually emanate from any aspect of the entity or not. Management of substrates could be achieved through the management of stimuli that modulate or moderate or influence any aspect of the substrate as well as through the management of any stimuli emanating from the substrate. The results enable a wide range of novel applications in a variety of fields with far-reaching implications. For example, the functional organization of many regions of the brain including the superior temporal cortex which is believed to play a critical role in the hierarchical processing of human visual and auditory stimuli is poorly understood. It is not known precisely which layer within which region of the brain is responsible for which aspect of visual or auditory processing. Simultaneous non-invasive acquisition of bio-signals representing contributions from multiple layers of neuronal populations within the brain could provide new insights leading to the resolution of many of these outstanding issues and provide a deeper understanding of the underlying physiological processes.

Conference 10076: High-Speed Biomedical Imaging and Spectroscopy II: Toward Big Data Instrumentation and Management

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10076-1, Session 1

High-sensitivity chemical imaging for biomedicine by stimulated Raman scattering microscopy *(Invited Paper)*

Wei Min, Columbia Univ. (United States)

Innovations in spectroscopy principles and microscopy technology have significantly impacted modern biology and medicine. While most of the contemporary bio-imaging modalities harness electronic transition, nuclear spin or radioactivity, vibrational spectroscopy has not been widely used yet. Here we will discuss an emerging chemical imaging platform, stimulated Raman scattering (SRS) microscopy, which can enhance the otherwise feeble spontaneous Raman eight orders of magnitude by virtue of stimulated emission. When coupled with stable isotopes (e.g., deuterium and 13C) or bioorthogonal chemical moieties (e.g., alkynes), SRS microscopy is well suited for probing in vivo metabolic dynamics of small bio-molecules which cannot be labeled by bulky fluorophores. Physical principle of the underlying optical spectroscopy and exciting biomedical applications such as imaging lipid metabolism, protein synthesis, DNA replication, protein degradation, RNA synthesis, glucose uptake, drug trafficking and tumor metabolism will be presented.

10076-2, Session 1

Melanin-targeted nonlinear microscopy for label-free molecular diagnosis and staging *(Invited Paper)*

Warren S. Warren, Duke Univ. (United States)

Visible absorption in tissue is dominated by a very small number of chromophores (hemoglobins and melanins) with broad optical spectra; for melanins in particular, the optical absorption spectrum is typically featureless. In addition, scattering limits penetration depth. As a result, the most common microscopy application by far is with excised tissue, which can be stained. However, nonlinear optical methods have the additional advantages of greater penetration depth and reduced sensitivity to scattering. Traditional nonlinear microscopy relies on mechanisms which produce light of a different color than the irradiating lasers, such as second harmonic generation or two photon induced fluorescence, and this contrast is sparse in biological issue without expressing or injecting different chromophores. Recently, stable laser sources and pulse shaping/pulse train modulation methods have made it possible to detect a much wider range of nonlinear molecular signatures, even at modest laser powers (much less than a laser pointer). Here we show the utility of a variety of such signatures (pump-probe, pulse-shaped stimulated Raman, cross-phase modulation) to quantitatively image the biochemical composition of transparent or pigmented tissue in a variety of applications, ranging from thin, unstained tissue sections to live knockout mice. The rich biochemical information provided by this method can be used as an indicator of melanocyte activity, which in turn (for example) reflects the status of melanocytic lesions. Comparisons with model systems (synthetic melanin nanoparticles, sepia melanin) and analysis of melanin degradation pathways in vivo have led to a quantitative understanding of the molecular basis of these changes.

10076-3, Session 1

High-speed stimulated Raman scattering microscopy for studying the metabolic diversity of motile Euglena gracilis

Yuta Suzuki, Yoshifumi Wakisaka, The Univ. of Tokyo (Japan); Osamu Iwata, Ayaka Nakashima, euglena Co., Ltd. (Japan); Takuro Ito, Keio Univ. (Japan); Misa Hirose, Ryota Domon, Mai Sugawara, Norimichi Tsumura, Chiba Univ. (Japan); Hiroshi Watarai, The Univ. of Tokyo (Japan); Tomoyoshi Shimobaba, Chiba Univ. (Japan); Kengo Suzuki, euglena Co., Ltd. (Japan); Keisuke Goda, The Univ. of Tokyo (Japan) and Univ. of California, Los Angeles (United States) and Japan Science and Technology Agency (Japan); Yasuyuki Ozeki, The Univ. of Tokyo (Japan)

Microalgae have gained much attention for their ability to produce biomaterials that are useful as food supplements, drugs, biodegradable plastics, and biofuels. Among such microalgae, Euglena gracilis has become a popular species by virtue of its capability of accumulating useful metabolites including paramylon and lipid. To produce these metabolites efficiently, it is essential to develop and optimize genetic transformation and culture conditions, for which chemically specific analysis of microalgal cells including E. gracilis plays a critical role in understanding and controlling cell-to-cell variations in response to external stress. However, conventional analytical tools such as spontaneous Raman scattering and fluorescence microscopy are not suitable for evaluating a diverse population of motile microalgae due to their labeling requirement and limited imaging speed. Here we demonstrate video-rate label-free metabolite imaging of live E. gracilis using stimulated Raman scattering (SRS) – an optical spectroscopic method for probing the vibrational signatures of molecules with orders of magnitude higher sensitivity than spontaneous Raman scattering. Our SRS's high-speed image acquisition (27 metabolite images per second) allowed for population analysis of live E. gracilis cells cultured under nitrogen-deficiency stress – a technique for promoting the accumulation of paramylon and lipid within the cell body. Furthermore, we conducted three-dimensional chemical imaging of each live E. gracilis cell only within 0.67 seconds. Thus, our SRS system's fast imaging capability enables us to quantify and analyze previously inaccessible cell-to-cell variations in the metabolite accumulation of a large population of motile E. gracilis cells under different culture conditions.

10076-4, Session 1

High-throughput selective excitation multiphoton multifoci microscopy for in vivo imaging

Yi Xue, Kalen Paul Berry, Christopher James Rowlands, Massachusetts Institute of Technology (United States); Yu Takiguchi, Massachusetts Institute of Technology (United States) and Hamamatsu Photonics K.K. (Japan); Peter T. C. So, Elly Nedivi, Massachusetts Institute of Technology (United States)

Neuroscience research requires optical imaging technology with high resolution and high speed for either structural or functional imaging. According to these requirements, we are developing a next-generation high-

throughput selective access multifocal multiphoton microscopy (saMMM) for real-time monitoring of sensory-driven synaptic activity. We use SLM as the key component to holographically generate 3D distributed spots. As a pilot, we have demonstrated that we can generate the required density of spots to illuminate a 300x300x15 μm3 volume. Since the 3D synapse map for the holographic targeting is created on a separate, high-resolution, multispectral single focus two-photon scanning system, we have added line-scan temporal focusing (line TF) to the holographic excitation system that is capable of fully mapping the within three minutes. This ensures maximal spatial co-registration between the synaptic structural scan and the holographic targeting based on the dendritic outline. Since the excitation spots for holographic excitation are distributed in 3D within a 15 um thick slab, to distinguish synapses located above one another on a dendrite, we engineered the emission point spread function by Laguerre-Gaussian phase plate to extend the depth of field as well as encode their axial location. Our first target is to monitor calcium signals from approximately 10,000 locations corresponding to all excitatory synapses of a single neuron with 100 ms temporal resolution.

10076-5, Session 1

Sparse sampling image reconstruction in Lissajous trajectory beam-scanning multiphoton microscopy

Garth J. Simpson, Purdue Univ. (United States)

Sparse sampling enables access to full volume mapping without substantial loss of inherent information content at rates that would otherwise result in data floods. In brief, each neuron typically extends across many pixels in any one field of view, such that the inherent information content can potentially be accessed through sampling of a smaller subset of pixels. However, the quality of the subsequent reconstruction depends greatly on the sampling pattern involved. In this work, Lissajous trajectories are shown to strike a balance between efficient sampling and the practical complexities associated with physical movement of the beam in a beam-scanning instrument. Lissajous scanning can be implemented on a common fast-scanning instrument combining a resonant scanner with a galvanometer, requiring only changes in the analysis of the recovered data. By integration of temporal multiplexing, multiple focal planes can be accessed from a single laser pulse in rapid succession, providing access to whole volumes at high speeds. Because the data are sampled sparsely, the total data throughput required for volume reconstruction corresponds to a fraction of what would be produced by brute-force approaches. Furthermore, the final volume outputs generated from the reconstruction can be similarly compressed to output files of manageable size. Examples for this approach are provided using model systems, with the benefits and limitations critically discussed.

10076-6, Session 2

Pushing the physical limits of in vivo spectroscopic imaging for new biology and better medicine *(Invited Paper)*

Ji-Xin Cheng, Purdue Univ. (United States)

In vivo molecular spectroscopic imaging is not a simple addition of a spectrometer to a microscope. Innovations are needed to break the physical limits in sensitivity, depth, speed and resolution perspectives. I will present our most recent advances in modality development, biological application, and clinical translation. My talk will focus on the development of mid-infrared photothermal microscope for depth-resolved vibrational imaging of living cells (Science Advances, in press), the discovery of a metabolic signature in cancer stem cells by hyperspectral stimulated Raman scattering imaging (Cell Stem Cell, in press), and the development of an intravascular vibrational photoacoustic catheter for label-free sensing of lipid laden plaques (Scientific Report 2016, 6:25236).

10076-7, Session 2

Ultrafast broadband Fourier-transform CARS spectroscopy operating at 50,000 spectra/second

Miu Tamamitsu, Yusuke Sakaki, Tasuku Nakamura, Gopala Krishna Podagatlapalli, Takuro Ideguchi, Keisuke Goda, The Univ. of Tokyo (Japan)

Coherent Raman scattering (CRS) spectroscopy is a useful tool in biochemistry and medicine as it offers the sample's intrinsic molecular vibrational information in a non-invasive, label-free manner. However, simultaneous achievement of both a high CRS spectral acquisition rate and a broad accessible Raman spectral bandwidth has been a difficult problem with conventional approaches. To overcome this hurdle, we propose and demonstrate a CRS spectroscopy method capable of acquiring 50,000 broadband CRS spectra/second. The method is based on a scanning Fourier-domain delay line serving as an optical path-length scanner in one of the two arms of a Michelson interferometer in a Fourier-transform CARS (FT-CARS) spectroscopy platform. The ultrafast spectral acquisition is realized by a rapidly rotating multi-faceted polygonal mirror array implemented as the scanning mechanism of the delay line, while the FT-CARS spectroscopy technique employing a broadband femtosecond laser allows for the broadband CARS spectral acquisition. In our proof-of-concept experiment, we implemented a 54-faceted polygonal mirror array rotating at 916 rounds/second and probed the liquid toluene sample with the constructed optical setup. The demonstration shows that the record CRS spectral acquisition rate of 50,000 spectra/second over most of the molecular fingerprint region (200 - 1430 cm-1) with a spectral resolution of 4.2 cm-1 is possible with the method. We expect that this high-speed, broadband and high-resolution CRS spectroscopy method would be of great use in applications where high-throughput screening or real-time monitoring of unknown samples with high specificity is required, such as single-cell analysis and biomedical imaging.

10076-8, Session 2

Optimization and applications of an excitation-scanning hyperspectral imaging system

Sam A. Mayes, Phiwat Klomkaew, Silas J. Leavesley, Thomas C. Rich, Univ. of South Alabama (United States)

Herein, we will describe the system and evaluate optimizations and applications of a novel excitation-scanning hyperspectral imaging system. Currently, the majority of microscopic and endoscopic technologies utilize white light illumination. For a number of applications, hyper-spectral imaging can be shown to have significant improvements over standard white-light imaging techniques. This is true for both microscopy and in vivo imaging. However, hyperspectral imaging methods have suffered from slow application times. Often, minutes are required to gather a full imaging stack. We have developed and are optimizing a novel approach called excitation-scanning hyperspectral imaging that provides an order of magnitude increased signal strength. Optimization of the light path, optical components and illumination sources have allowed us to achieve high speed image acquisition. This high speed allows for potential live video acquisition.

This excitation-scanning hyperspectral imaging technology has potential to impact a range of applications. The current system allows triggering of up to 16 wavelengths at less than 1 millisecond per image using digital strobing. Analog intensity control is also provided for a fully customizable excitation profile. A significant advantage of excitation-scanning hyperspectral imaging is can identify multiple targets simultaneously in real time. We are optimizing the system to compare sensitivity and specificity of excitation-scanning hyperspectral imaging with pathology techniques. Finally, we are exploring utilizing this technology to measure cAMP distribution in three dimensions within a cell.

10076-9, Session 2

Parallel-multiplexed excitation-emission light-sheet imaging in deep tissue

Dongli Xu, The Univ. of Arizona (United States); Weibin Zhou, Univ. of Michigan (United States); Leilei Peng, College of Optical Sciences, The Univ. of Arizona (United States)

Laser scanning light-sheet imaging allows fast 3D image of live samples with minimal bleach and photo-toxicity. Existing light-sheet techniques have very limited capability in multi-label imaging. Hyper-spectral imaging is needed to unmix commonly used fluorescent proteins with large spectral overlaps. However, the challenge is how to perform hyper-spectral imaging without sacrificing the image speed, so that dynamic and complex events can be captured live.

We report wavelength-encoded structured illumination light sheet imaging (?-SIM light-sheet), a novel light-sheet technique that is capable of parallel multiplexing in multiple excitation-emission spectral channels. ?-SIM light-sheet captures images of all possible excitation-emission channels in true parallel. It does not require compromising the imaging speed and is capable of distinguish labels by both excitation and emission spectral properties, which facilitates unmixing fluorescent labels with overlapping spectral peaks and will allow more labels being used together.

We build a hyper-spectral light-sheet microscope that combined ?-SIM with an extended field of view through Bessel beam illumination. The system has a 250-micron-wide field of view and confocal level resolution. The microscope, equipped with multiple laser lines and an unlimited number of spectral channels, can potentially image up to 6 commonly used fluorescent proteins from blue to red. Results from in vivo imaging of live zebrafish embryos expressing various genetic markers and sensors will be shown. Hyper-spectral images from ?-SIM light-sheet will allow multiplexed and dynamic functional imaging in live tissue and animals.

10076-10, Session 3

Real-time processing and analysis of gigapixel scale video *(Invited Paper)*

David J. Brady, Duke Univ. (United States)

No Abstract Available

10076-11, Session 3

High-speed imaging using compressed sensing and wavelength-dependent scattering

Jaewook Shin, Bryan T. Bosworth, Mark A. Foster, Johns Hopkins Univ. (United States)

The process of multiple scattering has inherent characteristics that are attractive for high-speed imaging with high spatial resolution and a wide field-of-view. A coherent source passing through a multiple-scattering medium naturally generates speckle patterns with diffraction-limited features over an arbitrarily large field-of-view. In addition, the process of multiple scattering is deterministic allowing a given speckle pattern to be reliably reproduced with identical illumination conditions. Here, by exploiting wavelength dependent multiple scattering and compressed sensing, we develop a high-speed 2D time-stretch microscope. Highly chirped pulses from a 90-MHz mode-locked laser are sent through a 2D grating and a ground-glass diffuser to produce 2D speckle patterns that rapidly evolve with the instantaneous frequency of the chirped pulse. To image a scene, we first characterize the high-speed evolution of the generated speckle patterns. Subsequently we project the patterns onto the microscopic region of interest and collect the total light from the scene using a single high-speed photodetector. Thus the wavelength dependent speckle patterns serve as high-speed pseudorandom structured illumination of the scene. An image sequence is then recovered using the time-dependent signal received by the photodetector, the known speckle pattern evolution, and compressed sensing algorithms. Notably, the use of compressed sensing allows for reconstruction of a time-dependent scene using a highly sub-Nyquist number of measurements, which both increases the speed of the imager and reduces the amount of data that must be collected and stored. We will discuss our experimental demonstration of this approach and the theoretical limits on imaging speed.

10076-12, Session 3

Fast single-pixel Fourier spectrum acquisition via temporal frequency-division multiplexing

Yuwang Wang, Jinli Suo, Tsinghua Univ. (China); Delei Chen, Beijing Institute of Technology (China); Wenhui Liu, Tsinghua Univ. (China) and Beijing Institute of Technology (China); Qionghai Dai, Tsinghua Univ. (China)

Single pixel imaging techniques have advantages compared to conventional CCD/CMOS array sensor imaging, such as wider spectral response range and looking around corner, et al. But poor imaging quality limits its wide application until Fourier spectrum acquisition method brings a large improvement in imaging quality due to its robust imaging reconstruct method. But this method needs to project large number of fourier patterns which results in low time efficient. In this paper, we present a single pixel fourier acquisition method based on Frequency-division Multiplexing strategy to speed up the acquisition process. Fourier patterns are spatially separated on fourier plane which enables us to modulate temporally changed intensity with different frequency for the different patterns. Thanks to the high speed temporal sampling frequency of single pixel detector, with the capture data of multiplexed patterns illuminated on the sample, we are able to reconstruct the results of each pattern illuminated on the sample respectively. As our simulation and real scene experiments shows, our method can speed up the process at least 8 times faster.

10076-19, Session 3

High-speed real-time image compression based on all-optical discrete cosine transformation

Qiang Guo, Hongwei Chen, Yuxi Wang, Minghua Chen, Sigang Yang, Shizhong Xie, Tsinghua Univ. (China)

Image compression techniques are designed to exploit the statistical redundancy present within real world images and have increased the capacity of information exchange in data-processing networks and communication systems. Single-pixel imaging is a novel optical image compression technique that enables image restoration with far fewer measurements than the number of reconstructed pixels. Recently, a single-pixel imaging scheme based on photonic time stretch is reported and its capability to perform image compression and capture fast dynamic phenomena are also demonstrated. However, the procedure for image reconstruction is an iterative process that consumes enormous amounts of computational time and cannot satisfy the requirement for real-time image transmission. To address this challenge, we propose an innovative optical image compression technique which can produce high-quality images by performing all-optical discrete cosine transform. A series of sinusoidal light patterns with pre-designed frequencies and initial phases are generated to illuminate the test samples and only the most significant cosine coefficients are acquired. Image reconstruction is completed by performing discrete cosine transform operations on the acquired measurements. A compression

ratio of approximately 10:1 and a fast image reconstruction procedure, which is two orders of magnitude faster than that of previous time-stretch-based single-pixel cameras, are both achieved in our prototype system, which implicates broad applications in industrial quality control and biomedical imaging.

10076-13, Session 4

Time of flight imaging through scattering environments

Toan H. Le, Illinois Wesleyan Univ. (United States); Eric C. Breitbach, Lab. for Optical and Computational Instrumentation, Univ. of Wisconsin-Madison (United States); Jonathan A. Jackson, Univ. of California, Berkeley (United States); Andreas Velten, Lab. for Optical and Computational Instrumentation, Univ. of Wisconsin-Madison (United States)

Light scattering is a primary obstacle to imaging in many environments. On small scales in biomedical microscopy and diffuse tomography scenarios scattering is caused by tissue. On larger scales scattering from dust and fog provide challenges to vision systems for self driving cars and naval remote imaging systems. We are developing scale models for scattering environments and investigation methods for improved imaging particularly using time of flight transient information.

With the emergence of Single Photon Avalanche Diode detectors and fast semiconductor lasers, illumination and capture on picosecond timescales are becoming possible in inexpensive, compact, and robust devices. This opens up opportunities for new computational imaging techniques that make use of photon time of flight.

Time of flight or range information is used in remote imaging scenarios in gated viewing and in biomedical imaging in time resolved diffuse tomography. In addition spatial filtering is popular in biomedical scenarios with structured illumination and confocal microscopy. We are presenting a combination analytical, computational, and experimental models that allow us develop and test imaging methods across scattering scenarios and scales. This framework will be used for proof of concept experiments to evaluate new computational imaging methods.

10076-14, Session 4

Label-free imaging of strongly scattering specimens using gradient light interference microscopy (GLIM) *(Invited Paper)*

Gabriel Popescu, Univ. of Illinois at Urbana-Champaign (United States)

Many specimens of interest, embryos, brain slices, organoids, are thick and optically inhomogeneous, which results in strong multiple scattering and low-contrast imaging. We developed GLIM as a novel label-free imaging method with applicability from nanoscale topographic structures to 2-300-micron thick tissues. Due to its interferometric principle that maintains equal power in the two interfering fields, GLIM is able to reject much of the multiple scattering background and generate high contrast interference. We present its principle and illustrate the performance with imaging nano pillars, cells, and embryos.

10076-16, Session 4

An efficient energy-density-dependent undersampling approach for compressive sensing in spectral domain optical coherence tomography

Sanjukta N. Bose, Jin U. Kang, Johns Hopkins Univ. (United States)

Many prior studies performed in the area of compressive optical coherence tomography (OCT) have mostly dealt with the problem of compressive sensing and sparse recovery of processed OCT images. Unlike these studies, in this paper, we study the application of compressive sensing in terms of efficient data storage and generating OCT images from undersampled raw unprocessed spectral domain OCT data. High resolution spectral domain OCT requires acquisition of enormous amount of data at very high sampling rate but such a large amount of the raw data impedes fast and efficient data storage and communication. To solve the problem of storing a large amount of data, we propose a specific undersampling method guided by the energy density of the spectral domain data in order to facilitate sparse representation of the raw data in terms of its salient frequency domain samples. This method takes into account not just the higher amplitude spectral data, as suggested in some previous studies but samples data based on nearly uniform distribution of energy over all the sampling intervals in the entire spectrum. Finally, we apply some state of the art sparse recovery methods involving L1 minimization to recover our desired high resolution images from the undersampled spectral domain data. We demonstrate the performance of our proposed scheme by comparing it with the recovery accuracy of some recent energy-guided undersampling methods and the conventional compressive sensing with random undersampling. We also compare the performance of our method with the other methods in terms of data compression ratio with respect to the reconstruction error.

10076-17, Session 4

Video-rate imaging using bandwidth-compressed subsampled optical coherence tomography

Meena Siddiqui, Harvard Medical School (United States); Norman Lippok, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States); Serhat Tozburun, Harvard Medical School (United States) and Wellman Ctr. for Photomedicine (United States); Ahhyun S. Nam, Massachusetts Institute of Technology (United States); Isabel Chico-Calero, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States); Reza Khazaeinezhad, Harvard Medical School (United States) and Wellman Ctr. for Photomedicine (United States); Martin Villiger, Benjamin J. Vakoc, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States)

We demonstrate video-rate and wide-field OCT imaging for the first time with a system that 1) utilizes a novel concept of optical compression and 2) operates at video rates. Structural volumetric videos of ex vivo swine GI tract and bladder and functional videos of rat sciatic nerve are presented to demonstrate the capability of the platform. At the core of this technology is a discretely-swept laser that features an optical comb spectral output, output speeds from 500 kHz to 19 MHz Aline rates, and coherence lengths of 3-7 cm.

10076-18, Session 4

AI-augmented time stretch microscopy

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Cell reagents used in biomedical analysis often change behavior of the cells that they are attached to, inhibiting their native signaling. However, label-free cell analysis techniques have long been viewed as challenging either due to insufficient accuracy by limited features, or because of low throughput as a sacrifice of improved precision. Here, we present a recently developed artificial-intelligence augmented microscope, which builds upon high-throughput quantitative time stretch imaging and deep learning to perform label-free cell classification with record high-accuracy. Our system captures quantitative optical phase and intensity images simultaneously by frequency multiplexing, extracts multiple biophysical features of the individual cells from these images fused, and feeds these features into a supervised machine learning model for classification. The enhanced performance of our system compared to other label-free assays is demonstrated by classification of white blood T-cells versus colon cancer cells and lipid accumulating algal strains for biofuel production, which is as much as five-fold reduction in inaccuracy. This system obtains the accuracy required in practical applications such as personalized drug development, while the cells remain intact and the throughput is not sacrificed.

10076-48, Session PMon

High-speed multicolor stimulated Raman scattering microscopy enabled by rapid wavelength switching

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Label-free microscopy techniques with chemical contrast are desirable for analyzing living cells without relying on exogenous contrast agents. However, their applications have been limited by their imaging speed because the fast physiological motion of living cells often causes motion artifacts. Stimulated Raman scattering (SRS) microscopy overcomes this problem by virtue of its high-speed label-free image acquisition with molecular vibrational contrast. While single-color SRS microscopy can image only at a certain vibrational frequency, multicolor SRS imaging allows for the acquisition of SRS images at different vibrational frequencies, which can then be used to simultaneously investigate the distributions of different intracellular molecules. Although the ability of multicolor SRS microscopy is well recognized, its shortest pixel dwell time is limited to 4 μs/pixel, which is an order of magnitude longer than that of single-color video-rate SRS microscopy. Here we demonstrate high-speed multicolor SRS imaging by rapid wavelength switching of laser pulses, which is realized by the use of an optical intensity modulator as a time gate, a diffraction grating, and four fiber delay lines. Using the developed system, we present four-color SRS imaging at an unprecedented pixel dwell time of 0.2 μs/pixel. Furthermore, we demonstrate motion-artifact-free multicolor SRS imaging of fast moving polymer beads and living cells. Our results firmly support that the method is a powerful tool for the label-free analysis of living cells in microbiology, oncology, plant science, and medicine.

10076-49, Session PMon

Using stroboscopic flow imaging to validate large-scale computational fluid dynamics simulations

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The utility and accuracy of computational modeling often requires direct validation against experimental measurements. The work presented here is motivated by taking a combined experimental and computational approach to determine the ability of large-scale computational fluid dynamics (CFD) simulations to understand and predict the dynamics of circulating tumor cells in clinically relevant environments. Experimentally, it is necessary to track the position of labeled cells or surrogate fluorescent beads over macroscopic distances under physiologically-relevant flow conditions. We use stroboscopic light sheet fluorescence imaging to track the paths and measure the velocities of fluorescent microspheres throughout a human aorta model. Bead positions are tracked with micrometer accuracy over 1-2 cm long tracks, moving at velocities up to 1-2 m/s, providing a critical reference point for CFD simulations. We discuss methods we have developed for performing light sheet imaging of beads, including the image analysis required to extract the bead velocities and tracks. Performed over complex physiologically –realistic 3D geometries, large data sets are acquired with microscopic resolution over macroscopic distances. The capability to acquire and analyze such large, challenging data sets will help drive progress in many areas of future biomedical science.

10076-50, Session PMon

Application of imaging flow cytometry for characterization of RBC morphology

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The morphological state of red blood cells (RBCs) can provide a strong indication of its efficacy viability; smooth/crenated discocytes (type I) possess the deformability and membrane integrity of an effective RBC, while crenated spheroids/spheres (type II) have undergone irreversible membrane degradation. To perform morphology characterization (MC) on a sample of RBCs, blood banks currently rely on microscope techniques that include fixing, staining and cell counting; these methods can be time-consuming and cumbersome when applied large samples. This study presents a novel method for RBC MC using an imaging flow cytometer (IFC).

IFC measurements were carried out on 8 bags of freshly donated (NetCad, CBS) packed RBCs; three independent 5 l samples were suspended in 200l PBS and then placed into the IFC (ImageStreamX Mark II, MilliporeSigma). Brightfield images of 100,000 objects were collected for each sample, and an image analysis software (IDEAS, MilliporeSigma) was used for segregation of RBC populations (type I and II). Samples were extracted and analysed every 2-7 days throughout the lifespan of the blood bags (6 weeks).

Typical final RBC images used for analysis ranged from 20,000-30,000; the acquisition/analysis of one sample was less than a half hour. Over the bag lifespan, type I and type II population declined and increased, respectively, by 37.11.57%. Variation within each IFC measurement ranged from 0.52-6.8%. Results demonstrate the potential of IFC image analysis to rapidly perform MC of RBCs; future steps will advance the method to include subtypes of RBC shapes typically used in morphological analysis.

10076-51, Session PMon

High-speed complete OCT signal processing solution

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High-speed real-time swept-source optical coherence tomography (SS-OCT) signal processing is a challenging task. Acquired signal often needs to be interpolated from constant time to constant wavenumber spacing. Windowing and dispersion compensation can then be performed followed by zero-padding if needed. The A-line is retrieved after a Fourier Transform and calculation of the logarithm of the amplitude. We present here a complete solution for signal acquisition up to 4 GS/s and on-board FPGA-based OCT processing at full speed.

To remove the need for interpolation, our boards offer variable frequency external clocks to use a linear-in-wavenumber clock (k-clock) from the laser. Since some k-clocks can be difficult to use as a sample trigger due to very narrow glitches or incomplete up-time, we have developed a module to configure the external clock sample to ignore the regions where the k-clock signal may generate errors. On-board OCT signal processing virtually eliminates the downstream signal processing bottleneck. Complex filtering allows for windowing and dispersion compensation. Data is zero-padded when the number of samples is not a power of 2. The FFT engine allows for up to 1,000,000 4096-point FFT calculations per second. Output data can be linear amplitude or logarithmic. This output data can be single-precision floating point or sliced to U32, U16 or U8. This hardware solution comes with dedicated software and software development kit.

10076-52, Session PMon

Separation of multi-exponential decaying constants in high-speed fluorescence lifetime imaging

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Analog mean delay method (AMD) is one of the fastest ways to measure fluorescence lifetimes in fluorescence lifetime imaging microscopy (FLIM). Its accuracy and photon economy have been demonstrated to be as good as those of time correlated single photon counting (TC-SPC) method. The major drawback of AMD method is its inherent difficulties in measuring two or more time constants within a multi-exponential decaying sample. Here we propose a way to distinguish two or more exponential decaying constants within a sample by using a proper deconvolution and numerical fitting schemes in the AMD method. We have demonstrated that two or more decaying time constants can be readily separated within a single measured waveform in a AMD-FLIM system. First, we measured the IRF of our system without a sample. Then, we carried out numerical simulations to find various effects of an AMD system such as the bandwidth or the digitizing resolution of a digitizer, response time of a detector, etc. on the accuracy of finding multi-exponential decaying constants. We have demonstrated the feasibility of our proposed measured by measuring the two decaying constants of a quantum dot made of Cdse/Zns - core/shell structure with 1.7 nm radius. We compared our results with those obtained with the TC-SPC method.

10076-54, Session PMon

Hyperspectral microscopy for tissue identification

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Oral cancer incidences have been increasing in recent years and late detection means that the prognosis is often poor. Raman spectroscopy has been identified has a valuable diagnostic tool but its time consuming nature has prevented its clinical use. In order to make Raman a realistic diagnostic aid to histopathology, a rapid pre-processing technique is required to find regions of interest. The feasibility of spectral scanning hyperspectral imaging for this purpose is investigated in this work. The current system can capture 450 focused, intensity-corrected and background-corrected images with wavelength ranging from 450-900 nm in around 40 minutes with spatial resolution of the order of microns and a spectral resolution of the order of 10 nm. Cluster analysis of hyperstacks of known absorbing samples, including fluorescent dyes, blood and stained tissue, have produced very good results with spectrally accurate transmission spectra and intensity variations with concentration. Using this technique, the presence of different components from a non-absorbent saliva droplet sample were successfully shown. Unstained oral tissue sections, however, were found to require segmentation and further analysis in order to separate different tissue types although tissue and non-tissue regions were clearly separated and some internal tissue features are highlighted. This has provoked investigation into other spectral analysis techniques and texture analysis of the morphological data.

10076-20, Session 5

Video-rate volumetric functional imaging of the brain at synaptic resolution *(Invited Paper)*

Na Ji, Howard Hughes Medical Institute (United States)

Understanding neural computation requires the ability to measure how a neuron integrates its synaptic inputs, and how neurons work together encoding sensory stimulation and executing motor control. With submicron lateral resolution and optical sectioning ability in scattering brains, two-photon laser scanning microscopy (2PLSM) has become a powerful tool for monitoring the activity of neurons and their networks in vivo. However, both individual neurons and neural networks may extend over hundreds or even thousands of micrometers in three dimensions. Because conventional 2PLSM images volume by scanning the excitation laser focus serially in 3D, the limited brightness of calcium indicators and the inertia of laser scanning units make it difficult to capture all the calcium transients in a volume at video rate (e.g., 30 Hz) while maintaining synapse-resolving ability.

Realizing that for most in vivo brain imaging experiments, the positions of neurons as well as their synapses are known and remain unchanged during each imaging session, one can increase volume imaging speed substantially by sacrificing axial resolution. By scanning an axially elongated focus (e.g., a Bessel beam) in 2D, we obtained projected views of 3D volumes and converted 2D frame rates into 3D volume rates. Utilizing a spatial light modulator, we built a module that generated Bessel foci

optimized for in vivo brain imaging. Easily incorporated into three different 2PLSM systems, this simple module allowed volume imaging rates of up to 30 Hz while maintaining the ability to resolve dendritic spines and axonal boutons. To demonstrate its versatility, we applied the Bessel focus scanning method to imaging brain volumes of a variety of model systems in vivo, such as fruit fly, zebrafish larva, mouse, and ferret. High speed volume imaging was used to inform on the tuning properties of dendritic spines and the synchrony of inhibitory neuron activity in mouse visual cortex, the network dynamics of reticulospinal neurons in the hindbrains of zebrafish larvae, and the responses of fruit fly brains to visual stimuli.

10076-21, Session 5

Feeling for cell function: Mechanical phenotyping at 1,000 cells/sec *(Invited Paper)*

Jochen R. Guck, TU Dresden (Germany)

The mechanical properties of cells have long been heralded as a label-free, inherent marker of biological function in health and disease. Wide-spread utilization has so far been impeded by the lack of a simple and convenient measurement technique with sufficient throughput. To address this need, we have introduced real-time deformability cytometry (RT-DC; Otto et al., Nat. Methods, 2015) for the continuous mechanical single-cell characterization of large populations (> 100,000 cells) with analysis rates up to 1,000 cells/s, approaching that of conventional fluorescence-based flow cytometers. Cells are flowed through a microfluidic constriction without contact to the walls at 10 cm/sec, deformed by hydrodynamic stresses, and the ensuing deformations are imaged and analysed in real-time. Analytical and numerical modelling of the hydrodynamic stress together with appropriate mechanical models allows the extraction of actual material properties (Mietke et al., Biophys. J., 2015). Even 1D fluorescence imaging and real-time analysis at three colours is possible at the same time. Using RT-DC we can sensitively detect cytoskeletal alterations, distinguish cell cycle phases, track hematopoietic stem cell differentiation into distinct lineages and characterize cell?populations in whole blood by their mechanical fingerprint. Our results indicate that cell mechanics can define cell function, can be used as an inherent cell marker and could serve as target for novel therapies. Mechanical phenotyping adds a new functional, marker-free dimension to flow cytometry with diverse applications in biology, biotechnology and medicine.

10076-22, Session 5

High-throughput label-free screening of euglena gracilis with optofluidic time-stretch quantitative phase microscopy

Baoshan Guo, Cheng Lei, Takuro Ito, Yasuyuki Ozeki, Keisuke Goda, The Univ. of Tokyo (Japan)

The development of reliable, sustainable, and economical sources of alternative fuels is an important, but challenging goal for the world. As an alternative to liquid fossil fuels, algal biofuel is expected to play a key role in alleviating global warming since algae absorb atmospheric CO2 via photosynthesis. Among various algae for fuel production, Euglena gracilis is an attractive microalgal species as it is known to produce wax esters (good for biodiesel and aviation fuel) within lipid droplets. To date, while there exist many techniques for inducing microalgal cells to produce and accumulate lipid with high efficiency, few analytical methods are available for characterizing a population of such lipid-accumulated microalgae including E. gracilis with high throughout, high accuracy, and single-cell resolution simultaneously. Here we demonstrate high-throughput, label-free, single-cell screening of E. gracilis with optofluidic time-stretch quantitative phase microscopy that can simultaneously provide both amplitude and phase images of E. gracilis cells at a high throughput of 10,000 cells/s. The phase information of E. gracilis is useful for identifying intracellular compounds such as lipids based on their refractive index contrast. Specifically, based on the amplitude and phase map of each cell, we classify nitrogen-sufficient (ordinary) and nitrogen-deficient (lipid-accumulated) E. gracilis cells in a non-invasive manner. This method holds promise for evaluating cultivation techniques and selective breeding for microalgae-based biofuel production. The microscope is also potentially applicable for identifying other cell types such as blood cells and cancer cells.

10076-23, Session 5

Recent advances in high-throughput QCL-based infrared microspectral imaging

Jeremy A. Rowlette, Edeline Fotheringham, David Nichols, Miles J. Weida, Justin Kane, Allen Priest, David B. Arnone, Benjamin Bird, William B. Chapman, David B. Caffey, Paul Larson, Timothy Day, Daylight Solutions Inc. (United States)

The field of infrared spectral imaging and microscopy is advancing rapidly due in large measure to the recent commercialization of the first high-throughput, high-spatial-definition quantum cascade laser (QCL) microscope. Having speed, resolution and noise performance advantages while also eliminating the need for cryogenic cooling, its introduction has established a clear path to translating the well-established diagnostic capability of infrared spectroscopy into clinical and pre-clinical histology, cytology and hematology workflows.

Demand for even higher throughput while maintaining high-spectral fidelity and low-noise performance continues to drive innovation in QCL-based spectral imaging instrumentation. In this talk, we will present for the first time, recent technological advances in tunable QCL photonics which have led to an additional 10X enhancement in spectral image data collection speed while preserving the high spectral fidelity and SNR exhibited by the first generation of QCL microscopes. This new approach continues to leverage the benefits of uncooled microbolometer focal plane array cameras, which we find to be essential for ensuring both reproducibility of data across instruments and achieving the high-reliability needed in clinical applications. We will discuss the physics underlying these technological advancements as well as the new biomedical applications these advancements are enabling, including automated whole-slide infrared chemical imaging on clinically relevant timescales.

10076-24, Session 5

High-throughput label-free platelet aggregate detection with optofluidic time-stretch microscopy

Yiyue Jiang, The Univ. of Tokyo (Japan); Cheng Lei, The Univ. of Tokyo (Japan) and Tsinghua Univ. (China); Atsushi Yasumoto, The Univ. of Tokyo (Japan); Takuro Ito, The Univ. of Tokyo (Japan) and Japan Science and Technology Agency (Japan); Baoshan Guo, Hirofumi Kobayashi, Yasuyuki Ozeki, Yutaka Yatomi, The Univ. of Tokyo (Japan); Keisuke Goda, The Univ. of Tokyo (Japan) and Japan Science and Technology Agency (Japan) and Univ. of Califonia, Los Angeles (United States)

According to WHO, approximately 10 million new cases of thrombotic disorders are diagnosed worldwide every year. In the U.S. and Europe, their related diseases kill more people than those from AIDS, prostate cancer, breast cancer and motor vehicle accidents combined. Although thrombotic disorders, especially arterial ones, mainly result from enhanced platelet aggregability in the vascular system, visual detection of platelet aggregates in vivo is not employed in clinical settings. Here we present a high-throughput label-free platelet aggregate detection method, aiming at the diagnosis and monitoring of thrombotic disorders in clinical settings. With optofluidic time-stretch microscopy with a spatial resolution of 780 nm and an ultrahigh linear scanning rate of 75 MHz, it is capable of detecting aggregated platelets in lysed blood which flows through a hydrodynamic-focusing microfluidic device at a high throughput of 10,000 particles/s. With digital image processing and statistical analysis, we are able to distinguish them from single platelets and other blood cells via morphological features. The detection results are compared with results of fluorescence-based detection (which is slow and inaccurate, but established). Our results

indicate that the method holds promise for real-time, low-cost, label-free, and minimally invasive detection of platelet aggregates, which is potentially applicable to detection of platelet aggregates in vivo and to the diagnosis and monitoring of thrombotic disorders in clinical settings. This technique, if introduced clinically, may provide important clinical information in addition to that obtained by conventional techniques for thrombotic disorder diagnosis, including ex vivo platelet aggregation tests.

10076-25, Session 6

Microscope add-on kits for whole slide imaging and 3D confocal sectioning *(Invited Paper)*

Guoan Zheng, Univ. of Connecticut (United States)

Fluorescence microscopy is a standard choice for many imaging applications in basic biomedical research. A critical consideration for high-throughput whole slide imaging (WSI) platform is to perform accurate autofocusing in high speed. Commercial WSI systems acquire a z stack of sample images and determine the best focal position by maximizing certain figure of merits. This strategy, however, suffer from several drawbacks: 1) low speed due to multiple image acquisitions, 2) low accuracy of focal plane estimation, 3) a short z range for autofocusing, and 4) difficulties for handling unstained or low-contrast samples. By exploring the image correlation property of tissue sections, we report a single-frame ultrafast autofocusing scheme to address the above challenges. We demonstrate the use of the reported autofocusing scheme for unstained transparent samples. The average autofocusing error using the reported scheme is ~3 folds better than that of commercial systems. The reported scheme may find applications in high-throughput WSI and DNA-sequencing systems. In the second part of the talk, we will report the development of an add-on kit for high-speed and low-cost confocal microscopy. By adapting this add-on kit to an existing regular microscope, one can convert it into a confocal microscope without significant hardware modifications. Compared with current projector-based implementations, the reported approach is able to recover multiple layers along the z axis simultaneously. It may find applications in wafer inspection and 3D metrology of semiconductor circuit. The dissemination of the proposed add-on kit under $1000 budget could also lead to new types of experimental designs for biological research labs.

10076-26, Session 6

Photonics and high-throughput techniques: to relieve the bottleneck of plant functional genomics

Qian Liu, Huazhong Univ. of Science and Technology (China); Wanneng Yang, Huazhong Agricultural Univ. (China); Xiong Xiong, Huazhong Univ. of Science and Technology (China); Lizhong Xiong, Huazhong Agricultural Univ. (China)

Plant phenomics has now become a new bottleneck in plant science. Driven by the phenotyping bottleneck, many researches use non-invasive technologies such as optical imaging and image analysis to inspect plant growth and architecture, especially in the field. However, high-throughput, large-scale, and automated phenotyping platforms for controlled in-door environments are solely lacking. This research presented an automated rice phenotyping platform for measurement and evaluation of key phenotypic traits required in analyzing, ranking, and selecting valuable germplasm for pot-grown rice plant. The platform mainly includes two systems: rice plant phenotying system (RAP) and yield traits scorer (YTS). During growth, rice plants are automated transported to the imaging chamber of rice plant phenotying system via industrial conveyor. The system integrated three imaging technologies in one imaging chamber: multi-angle visible light imaging for measuring plant height, biomass and leaf area, X-ray computed tomography for measuring tiller number, and thermal imaging for measuring canopy temperature. After harvest, rice spikelets were inputted into the seed traits scorer to evaluate yield traits including total spikelet number, filled spikelet number, grain length, grain width, and thousand grain weight. The tests showed that, correlation coefficients between automated measurements and manual measurements were greater than 0.9 for all the extracted traits. Measuring efficiency was 30 seconds per plant and 2 minutes per plant for rice plant phenotying system and seed traits scorer, respectively. Integrated with genome wide association studies (GWAS), this platform would be a powerful tool in accelerating rice genomic research and rice breeding.

10076-27, Session 6

Scheimpflug multi-aperture Fourier ptychography: coherent computational microscope with gigapixels/s data acquisition rates using 3D printed components

Pavan Chandra Konda, Jonathan M. Taylor, Andrew Robert Harvey, Univ. of Glasgow (United Kingdom)

Obtaining gigapixel images at high frame rates is a challenging task because of the aberrations typically present in conventional optical systems, small sensor sizes and slow data capture rates of the cameras. Multi-Aperture Fourier Ptychography (MAFP) was proposed recently by us to solve the issue of low data acquisition bandwidths in Fourier Ptychography (FP), a technique to obtain wide field-of-view, large Depth-of-field, high resolution images by trading data acquisition time. This is achieved by parallelizing data capture using an array of lenses coupled with detectors thus enabling data acquisition rates in gigapixels/s. Here we present an advanced MAFP system based on Scheimpflug configuration which has better performance at high NAs. This system requires a complicated optical setup because of its large number of degrees of freedom (6 times the number of lenses). Hence we developed a 3D printed system which solves this issue and decreases the cost of the setup significantly. The lens positions are fixed by 3D printed mounts hence reducing 3 degrees of freedom for each lens system. A mechanical stage with tip, tilt and translation capabilities is designed using 3D printed parts and fine screws to mount the detector allowing it 3 degrees of alignment with high-precision. All the components are assembled together using metal posts mounted into the 3D printed parts to reduce thermal drift. We will describe Scheimpflug MAFP system principles and present our 3D printed setup designs along with experimental validation.

10076-28, Session 6

Label-free image-based detection of drug resistance with optofluidic time-stretch microscopy

Hirofumi Kobayashi, Cheng Lei, Ailin Mao, Yiyue Jiang, Baoshan Guo, Yasuyuki Ozeki, Keisuke Goda, The Univ. of Tokyo (Japan)

Acquired drug resistance is a fundamental predicament in cancer therapy. Early detection of drug-resistant cancer cells during or after treatment is expected to benefit patients from unnecessary drug administration and thus play a significant role in the development of a therapeutic strategy. However, the development of an effective method of detecting drug-resistant cancer cells is still in its infancy due to their complex mechanism in drug resistance. To address this problem, we propose and experimentally demonstrate label-free image-based drug resistance detection with optofluidic time-stretch microscopy using leukemia cells (K562 and K562/ADM). By adding adriamycin (ADM) to both K562 and K562/ADM (ADM-resistant K562 cells) cells, both types of cells express unique morphological changes, which are subsequently captured by an optofluidic time-stretch microscope. These

unique morphological changes are extracted as image features and are subjected to supervised machine learning for cell classification. We hereby have successfully differentiated K562 and K562/ADM solely with label-free images, which suggests that our technique is capable of detecting drug-resistant cancer cells. Our optofluidic time-stretch microscope consists of a time-stretch microscope with a high spatial resolution of 780 nm at a 1D frame rate of 75 MHz and a microfluidic device that focuses and orders cells. We compare various machine learning algorithms as well as various concentrations of ADM for cell classification. Owing to its unprecedented versatility of using label-free image and its independency from specific molecules, our technique holds great promise for detecting drug resistance of cancer cells for which its underlying mechanism is still unknown or chemical probes are still unavailable.

10076-29, Session 7

Novel spirocyclization-based fluorogenic probes: From rapid intraoperative imaging of tiny tumors to super-resolution imaging *(Invited Paper)*

Yasuteru Urano, The Univ. of Tokyo (Japan)

We found that hydroxymethyl rhodamine green (HMRG) strongly fluoresces, while mono-amidated HMRG derivatives are colorless and non-fluorescent due to the preferred spirocyclized structure in aqueous solution at pH 7.4. Based on these findings, we have established a rational design strategy for novel fluorogenic probes, and developed various aminopeptidase-sensitive probes which were applicable for living cell system, including gGlu-HMRG, a novel HMRG-based “activatable” fluorescence probe for gamma-glutamyltranspeptidase (GGT). We could detect tiny tumors in vivo by spraying gGlu-HMRG onto tissue surfaces that are suspected of harboring tumors, creating high signal contrast between the tumor and the background within 1 min. Based on the same strategy of spirocyclization, we also succeeded to develop a first-in-class spontaneously blinking fluorophore for single-molecule localization microscopy (SLM) imaging to construct super-resolution images, by optimization of intramolecular nucleophile and rhodamine-based fluorophore. By taking advantage of the capability of spontaneous blinking regardless of laser intensity, SLM of nuclear pore structures far above from coverslip under spinning-disk confocal microscopes and repetitive time-lapse super-resolution imaging of microtubules in live cells could be achieved. Thus, our precisely designed spontaneously blinking fluorophore has a potential to expand the application of SLM deep into the cells and to track the motion of certain structure in living cells.

10076-30, Session 7

A high performance multi-tap CMOS lock-in pixel image sensor for biomedical applications

Min-Woong Seo, Yuya Shirakawa, Keiichiro Kagawa, Keita Yasutomi, Shoji Kawahito, Shizuoka Univ. (Japan)

Time-resolved techniques are widely used for various applications in life sciences and biomolecular interactions observation. The time-resolved applications require a detector that is fast, as determined by the time-constant of the impulse response, and sensitive, as determined by the minimum detectable signal level. To develop a detector with high charge modulation speed and high sensitivity, a lateral electric field charge modulation (LEFM) method and low-noise imaging techniques are applied to the proposed CMOS lock-in pixel. Furthermore, the pixel has two in-pixel storage diodes (SDs) with a large full well capacity (FWC), and it helps to improve the signal to noise ratio (SNR) of imager. The sensor, which is implemented with pinned photodiode 0.11?m 1-poly 4-metal CMOS technology, consists of the proposed lock-in pixel array, inverter tree for reducing the clock skew variations and clock drivers at the end of the inverter tree, column-parallel low-noise readout circuits, and vertical/horizontal scanner. To reduce the influence of parasitic resistance and capacitance, the driver array for LEFM is arranged at the upper and lower sides of the lock-in pixel array. As a result, a fast intrinsic response and low noise performance are achieved and demonstrated. The sensor is employed for a fluorescence lifetime imaging microscopy (FLIM) as a practical approach, and it has been experimentally implemented. The prototype multi-tap CMOS lock-in pixel sensor has an effective pixel array of 130(H)?256(V) and a pixel size of 11.2?m?11.2?m. The developed image sensor will be useful for bio and medical applications that require both high sensitivity and portability of the imaging system.

10076-31, Session 7

Random addressed full-field Fourier-transform spectrometry (RAFFS)

Dushan N. Wadduwage, Massachusetts Institute of Technology (United States)

Imaging Fourier transform spectrometry (IFTS) can be used for hyperspectral imaging in wide-field mode. For wide-field hyperspectral imaging, IFTS uniquely offers compressive data acquisition capabilities in the spectral dimension. Spectral properties can be compressively measured without measuring the complete spectrum due to the fact that each measurement contains partial information about the entire spectrum. However due to the limitations of the existing imaging interferometers this idea cannot be implemented in wide-field mode. Michelson interferometer is non-common-path and less stable and the stable common-path Sagnac interferometer is limited in the field of view due its phase tilt. Overcoming these limitations, in this work, we introduce a full-field imaging interferometer that can take advantage of compressive spectral recovery for a mixture of florescent molecules in biological specimens. We first simulate the compressive data acquisition scheme for a set of quantum dots and evaluate the expected system performance to measure properties of spectra. Then we demonstrate the same in an experiment to measure the peak-emission wavelengths for a fluorescent beads sample and for a mouse muscle tissue specimen with multiple species.

10076-32, Session 7

Scan-less confocal phase imaging with dual comb microscopy

Eiji Hase, Takeo Minamikawa, Shuji Miyamoto, The Univ. of Tokushima (Japan) and Japan Science and Technology Agency (Japan); Hirotsugu Yamamoto, Utsunomiya Univ. (Japan) and Japan Science and Technology Agency (Japan); Takeshi Yasui, The Univ. of Tokushima (Japan) and Japan Science and Technology Agency (Japan)

Optical frequency comb (OFC) has attracted attentions for high accuracy, high resolution, and broadband spectroscopy in visible and infrared regions because the mode-resolved OFC spectrum can be used as a precise frequency ruler due to both characteristics of broadband radiation and narrow-line CW radiation. However, application fields of OFC other than spectroscopy are still undeveloped. One interesting aspect of OFC except for the frequency ruler is optical carrier having a huge number of discrete frequency channels in the broad spectral range. If a physical quantity to be measured is encoded on each comb mode by dimensional conversion, a huge number of data for the measured quantity can be simultaneously obtained from a single mode-resolved spectrum of OFC.

In this study, we proposed a novel application of OFC, termed scan-less dual comb microscopy. In this approach, the confocal 2D image of a sample was encoded on OFC spectrum by using the 2D spectral disperser, and then the image-encoded OFC spectrum is acquired by dual comb spectrometer (DCS) to decode the 2D image of the sample. The combination of OFC with

the spectral encoding enables us to establish both confocality and full-field imaging under the scan-less condition. Furthermore, since DCS can provide the phase spectrum as well as the amplitude spectrum, the confocal volume of the sample can be precisely resolved along the depth axis by use of the phase spectrum. We demonstrated a proof-of-principle experiment of the proposed method by confocal phase imaging of a test chart.

10076-33, Session 8

Imaging single cells in a beam of live cyanobacteria with an x-ray laser *(Invited Paper)*

Gijs van der Schot, Tomas Ekeberg, Janos Hajdu, Uppsala Univ. (Sweden)

Imaging live cells at a resolution higher than achieved using optical microscopy is a challenge. Ultra-fast coherent diffractive imaging with X-ray free-electron lasers (XFELs) has the potential to achieve sub-nanometer resolution on micron-sized living cells. Our container-free injection method can introduce a beam of live cyanobacteria into the micron-sized focus of the Linac Coherent Light Source (LCLS) to record of diffraction patterns from individual cells, with very low noise at high hit rates (millions of cells/day). We used iterative phase retrieval to derive two-dimensional projection images directly from the diffraction patterns. In a first experiment, we collected diffraction patterns to 33-46 nm full-period resolution, and reconstructed the exit wave front to 76 nm full-period resolution. In a second experiment, we demonstrate that it is indeed possible to record diffraction data to nanometer resolution on live cells with an intense, ultra-short X-ray pulse as predicted earlier. These results are encouraging, and future developments to the XFELs and improvements to the X-ray area-detectors will bring sub-nanometer resolution reconstructions of living cells within reach. Utilizing this type of diffraction data will require the development of new analysis methods and algorithms for studying structure and structural variability in large populations of cells and to create abstract models. Such studies will allow us to understand living cells and populations of cells in new ways.

10076-34, Session 8

High-precision and large range one-shot 3D imaging with chirped fiber-based optical frequency comb *(Invited Paper)*

Kaoru Minoshima, Takashi Kato, Megumi Uchida, The Univ. of Electro-Communications (Japan) and Japan Science and Technology Agency (Japan)

We propose a method for one-shot 3D shape measurement using pulse-to-pulse spectral interferometry with a chirped optical frequency comb, which realizes high-precision, long range, and ultrafast time-resolution simultaneously. Simultaneous times-of-flight from multiple positions to a target can be obtained using an ultrafast conversion between space, time, and frequency information encoded in chirped ultrashort pulses. Fiber-based optical frequency comb provides practical compact measurement system with high stability and quality. We experimentally demonstrated a profile measurement of a known step height of 480 μm with μm-level accuracy with one-shot imaging. The measured step height agrees well with the nominal value. Furthermore, using the accurate pulse-to-pulse separation of the optical frequency comb, it is possible to extend the measurement range without losing the uncertainty, allowing for the measurement of a large step height of 3-m in one shot. Consequently, the distance between two mirrors was measured to be 2.9354928 m with a 9.8-μm uncertainty without having to scan the delay or beam position. Current measurement uncertainty was determined by the spectral non-uniformity of the light source, the optical setup and the evaluation system, all of which could be further improved. With further averaging along the beam position, high-accuracy measurement with 1-μm uncertainty was demonstrated.

The proposed method with great dynamic range and versatility of the measurements can naturally be extended to broad range of applications including microscopic structures, objects with large size or large aspect ratio, and ultrafast time-resolved imaging.

10076-35, Session 8

High-speed quantitative phase imaging using time-stretch spectral shearing contrast

Bryan Bosworth, Mark A. Foster, Johns Hopkins Univ. (United States)

Photonic time-stretch microscopy (TSM) provides an ideal platform for high-throughput imaging flow cytometry, affording extremely high shutter speeds and frame rates with high sensitivity. In order to resolve weakly scattering cells in biofluid and solve the issue of signal-to-noise in cell labeling specificity of biomarkers in imaging flow cytometry, several quantitative phase (QP) techniques have recently been adapted to TSM. However, these techniques have relied primarily on sensitive free-space optical configurations to generate full electric field measurements. The present work draws from the field of ultrashort pulse characterization to leverage the coherence of the ultrashort optical pulses integral to all TSM systems in order to do self-referenced single-shot quantitative phase imaging in a TSM system. Self-referencing is achieved via spectral shearing interferometry in an exceptionally stable and straightforward Sagnac loop incorporating an electro-optic phase modulator and polarization-maintaining fiber that produce sheared and unsheared copies of the pulse train with an inter-pulse delay determined by polarization mode dispersion. The spectral interferogram then yields a squared amplitude and a phase derivative image that can be integrated for conventional phase. We apply this spectral shearing contrast microscope to acquire QP images on a high-speed flow microscope at 90-MHz line rates with >400 pixels per line. We also consider the extension of this technique to compressed sensing (CS) acquisition by intensity modulating the interference spectra with pseudorandom binary waveforms to reconstruct the images from a highly sub-Nyquist number of random inner products, providing a path to even higher operating rates and reduced data storage requirements.

10076-36, Session 8

Optical time-stretch microscopy enabled by free-space angular-chirp-enhanced delay

Jianglai Wu, Yiqing Xu, Andy K. S. Lau, Anson H. L. Tang, Antony C. S. Chan, Kenneth K. Y. Wong, Kevin K. Tsia, The Univ. of Hong Kong (Hong Kong, China)

Optical time-stretch microscopy enables cellular images captured at tens of MHz line-scan rate and becomes a potential tool for ultrafast dynamics monitoring and high throughput screening in scientific and biomedical applications. In time-stretch microscopy, to achieve the fast line-scan rate, optical fibers are used as the pulse-stretching device that maps the spectrum of a light pulse to a temporal waveform for fast digitization. Consequently, existing time-stretch microscopy is limited to work at telecom windows (e.g. 1550 nm) where optical fiber has significant pulse-stretching and small loss. This limitation circumscribes the potential application of time-stretch microscopy.

Here we present a new optical time-stretch imaging modality by exploiting a novel pulse-stretching technique, free-space angular-chirp-enhanced delay (FACED), which has three benefits: (1) Pulse-stretching in FACED generates substantial, reconfigurable temporal dispersion in free-space with low intrinsic loss at visible wavelengths; (2) Pulse-stretching in FACED inherently provides an ultrafast all-optical laser-beam scanning mechanism for time-stretch imaging. (3) Pulse-stretching in FACED can be wavelength-

invariant, which enables time-stretch microscopy implemented without spectral-encoding.

Using FACED, we demonstrate optical time-stretch microscopy with visible light (~700 nm). Compared to the prior work, bright-field time-stretch images captured show superior contrast and resolution, and can be effectively colorized to generate color time-stretch images. More prominently, accessing the visible spectrum regime, we demonstrate that FACED enables ultrafast fluorescence time-stretch microscopy. Our results suggest FACED could unleash a wider scope of applications that were once forbidden with the fiber based time-stretch imaging techniques.

10076-37, Session 8

Ultrafast imaging of light scattering dynamics using the second-generation compressed ultrafast photography

Jinyang Liang, Cheng Ma, Liren Zhu, Yujia Chen, Washington Univ. in St. Louis (United States); Liang Gao, Univ. of Illinois at Urbana-Champaign (United States); Lihong V. Wang, Washington Univ. in St. Louis (United States)

Although light propagation in a scattering medium has been modeled by a number of simulation tools (such as Monte Carlo, finite-element, and finite-difference time-domain methods), conventional experimental instruments (such as CCD and CMOS sensors) still lack enough imaging speed to capture light scattering dynamics in real time. The streak camera is not restrained by the speed limit of conventional imaging sensors. However, conventionally, the streak camera allowed only one-dimensional ultrafast imaging. Ultrafast two-dimensional imaging requires additional scanning, posing an inherent challenge for moving scattering samples.

To overcome these limitations, we present single-shot real-time video recording of light scattering dynamics using second-generation compressed ultrafast photography (CUP). Specifically, we analytically derived the evolution of light intensity distribution in a thin scattering plate assembly using scattering theory and simulated it using the time-resolved Monte Carlo method. We experimentally captured this dynamic scene using second-generation CUP at 100 billion frames per second in a single camera exposure. Second-generation CUP, which implements a more efficient hardware design and a new reconstruction paradigm, largely improves the reconstructed image quality. Ultrafast imaging reveals the instantaneous light scattering pattern hidden from conventional time-integrated measurement. Our experimental results are in excellent agreement with the theoretical prediction. We envision that our technology will find a diverse range of applications in biomedical imaging, materials science, and physics.

10076-38, Session 8

High speed fluorescence imaging with compressed ultrafast photography

Jonathan V. Thompson, John D. Mason, Texas A&M Univ. (United States) and Air Force Research Lab. (United States); Hope Thomas Beier, Joel N. Bixler, Air Force Research Lab. (United States)

Fluorescent lifetime imaging is an optical technique that facilitates imaging molecularinteractions and cellular functions. Because the excited lifetime of a fluorophore is sensitive to its local microenvironment, measurement of fluorescent lifetimes can be usedto accurately detect regional changes in temperature, pH, and ion concentration. However, typical state of the art fluorescent lifetime methods are severely limited when it comes to acquisition time (on the order of seconds to minutes) and video rate imaging. Here we show that compressed ultrafast photography (CUP) can be used in conjunction with fluorescent lifetime imaging to overcome these acquisition rate limitations.

Frame rates up to one hundred billion frames per second have been demonstrated using compressed ultrafast photography with a streak camera. These rates are achieved by encoding time in the spatial direction with a pseudo-random binary pattern. The time domain information is then reconstructed using a compressed sensing algorithm, resulting in a cube of data (x,y,t) for each readout image. Thus, application of compressed ultrafast photography will allow us to acquire an entire fluorescent lifetime image with a single laser pulse. Using a streak camera with a high speed CMOS camera, acquisition rates of 100 frames per second can be achieved, which will significantly enhance our ability to quantitatively measure complex biological events with high spatial and temporal resolution. In particular, we will demonstrate the ability of this technique to do single-shot fluorescent lifetime imaging of cells and microspheres.

10076-39, Session 9

Large scale superres 3D imaging: light-sheet single-molecule localization microscopy *(Invited Paper)*

Chieh Han Lu, Peilin Chen, Bi-Chang Chen, Research Ctr. for Applied Sciences - Academia Sinica (Taiwan)

Optical imaging techniques provide much important information in understanding life science especially cellular structure and morphology because “seeing is believing”. However, the resolution of optical imaging is limited by the diffraction limit, which is discovered by Ernst Abbe, i.e. ?/2(NA) (NA is the numerical aperture of the objective lens). Fluorescence super-resolution microscopic techniques such as Stimulated emission depletion microscopy (STED), Photoactivated localization microscopy (PALM), and Stochastic optical reconstruction microscopy (STORM) are invented to have the capability of seeing biological entities down to molecular level that are smaller than the diffraction limit (around 200-nm in lateral resolution). These techniques do not physically violate the Abbe limit of resolution but exploit the photoluminescence properties and labelling specificity of fluorescence molecules to achieve super-resolution imaging. However, these super-resolution techniques limit most of their applications to the 2D imaging of fixed or dead samples due to the high laser power needed or slow speed for the localization process. Extended from 2D imaging, light sheet microscopy has been proven to have a lot of applications on 3D imaging at much better spatiotemporal resolutions due to its intrinsic optical sectioning and high imaging speed. Herein, we combine the advantage of localization microscopy and light-sheet microscopy to have super-resolved cellular imaging in 3D across large field of view. With high-density labeled spontaneous blinking fluorophore and wide-field detection of light-sheet microscopy, these allow us to construct 3D super-resolution multi-cellular imaging at high speed (~minutes) by light-sheet single-molecule localization microscopy.

10076-40, Session 9

Three-dimensional spatiotemporal focusing of holographic patterns *(Invited Paper)*

Valentina Emiliani, Oscar Hernandez, Eirini I. Papagiakoumou, Dmitrii Tanese, Emiliano Ronzitti, Univ. Paris Descartes (France); Kevin Fidelin, Institut National de la Santé et de la Recherche Médicale (France) and Univ. Pierre et Marie Curie (France); Claire Wyart, Institut National de la Santé et de la Recherche Médicale (France) and Institut du Cerveau et de la Moelle Épinière (France)

Genetically encoded light-sensitive channels and reporters enable both neuronal activity optical control and read-out. Full explotation of these optogenetic tools requires single-cell scale methods to pattern light into neural tissue.

Computer Generated Holography (CGH) can powerfully enhance optogenetic stimulation by efficiently shaping light onto multiple cellular targets. However, a linear proportionality between lateral shape area and axial extent degrades axial precision for cases demanding extended lateral patterning i.e., to cover entire soma of multiple cells. To address this limitation, we previously combined CGH with temporal focusing (TF) to stretch laser pulses outside of the focal plane, which combined with two-photon's nonlinear fluorescence dependence, axially confines fluorescence regardless of lateral extent. However, this configuration restricts nonlinear excitation to a single spatiotemporal focal plane: which is the objective focal plane.

Here we demonstrate a novel scheme enabling generation of spatiotemporally focused pattern generation in three dimensions. We demonstrate that this approach enables simultaneous photoconversion of tens of zebrafish larvae spinal cord neurons occupying separate axial planes.

10076-42, Session 9

Three-dimensional fluorescence imaging of particles in a glass capillary using tilted light-sheet illumination

Masaya Okada, Shigeki Iwanaga, Sysmex Corp. (Japan)

Flow cytometry has been widely used in clinical inspections such as blood cell counting due to its capability of high-speed counting and categorizing of cells by size and/or fluorescence properties. Further analysis of individual cellular properties and functions such as protein localization or gene distribution is expected to open up new clinical inspections like liquid biopsy. Recently, molecular imaging flow cytometer (MI-FCM), which can obtain shapes and/or molecular distributions of individual cells, has been developed as a next generation flow cytometer. However, since a cell has three-dimensional structure, three-dimensional observation is required for more precise analysis.

In order to develop a practical three-dimensional MI-FCM, we have designed a new optical layout, which uses light-sheet illumination for particles in a glass capillary. The path of illuminating light and detection path were arranged orthogonal to the length of the capillary and consequently, to the flow direction of flow cytometer. The light-sheet illumination was produced by expanding a CW laser in one direction only, with a cylindrical lens. Furthermore, the light-sheet illumination was tilted against the illumination axis to image a projection of a section of the sample by a CCD camera in the detection path. Fluorescence beads embedded within gels in the glass capillary were observed with this layout. Different sections of the sample were imaged through scanning the capillary itself along its length. By stacking inverse transformation of the projected two-dimensional images, the three-dimensional distribution of the fluorescence beads in the glass capillary was imaged.

10076-43, Session 9

Volumetric optical coherence microscopy enabled by aberrated optics

Jeffrey A. Mulligan, Siyang Liu, Steven G. Adie, Cornell Univ. (United States)

Optical coherence microscopy (OCM) is an interferometric imaging technique that enables high resolution, non-invasive imaging of 3D cell cultures and biological tissues. Volumetric imaging with OCM suffers a trade-off between high transverse resolution and poor depth-of-field resulting from defocus, optical aberrations, and reduced signal collection away from the focal plane. While defocus and aberrations can be compensated with computational methods such as interferometric synthetic aperture microscopy (ISAM) or computational adaptive optics (CAO), reduced signal collection must be physically addressed through optical hardware. Axial scanning of the focus is one approach, but comes at the cost of longer acquisition times, larger datasets, and greater image reconstruction times.

Given the capabilities of CAO to compensate for general phase aberrations, we present an alternative method to address the signal collection problem without axial scanning by using intentionally aberrated optical hardware. We demonstrate the use of an astigmatic spectral domain (SD-)OCM imaging system to enable single-acquisition volumetric OCM in 3D cell culture over an extended depth range, compared to a non-aberrated SD-OCM system. The transverse resolution of the non-aberrated and astigmatic imaging systems after application of CAO were 2 um and 2.2 um, respectively. The depth-range of effective signal collection about the nominal focal plane was increased from 100 um in the non-aberrated system to over 300 um in the astigmatic system, extending the range over which useful data may be acquired in a single OCM dataset. We anticipate that this method will enable high-throughput cellular-resolution imaging of dynamic biological systems over extended volumes.

10076-53, Session 9

High-speed three-dimensional fluorescence imaging with frequency-division-multiplexed confocal microscopy

Hideharu Mikami, Jeffrey Harmon, Yasuyuki Ozeki, The Univ. of Tokyo (Japan); Keisuke Goda, The Univ. of Tokyo (Japan) and Univ. of California, Los Angeles (United States) and Japan Science and Technology Agency (Japan)

We demonstrate high-speed three-dimensional fluorescence microscopy at a record high volume rate of over 100 volumes/sec. This is enabled by frequency-division-multiplexed confocal microscopy by which a focal spot array of excitation light is scanned by an 8,000-Hz resonant scanner, resulting in a two-dimensional frame rate of 16,000 frames/sec. We also use a piezoelectric objective scanner operating at > 50 Hz to obtain stacked two-dimensional images. In our proof-of-concept demonstration, we show three-dimensional imaging of 6-?m fluorescent beads at a volume rate of 100 volumes/sec. Our technique holds promise for diverse biomedical applications such as three-dimensional fluorescence imaging cytometry and real-time observation of fast dynamics in living organisms.

10076-44, Session 10

Holographic femtosecond laser processing and its application to biological materials *(Invited Paper)*

Yoshio Hayasaki, Utsunomiya Univ. (Japan)

Femtosecond laser processing is a promising tool for fabricating novel and useful structures on the surfaces of and inside materials. An enormous number of pulse irradiation points will be required for fabricating actual structures with millimeter scale, and therefore, the throughput of femtosecond laser processing must be improved for practical adoption of this technique. One promising method to improve throughput is parallel pulse generation based on a computer-generated hologram (CGH) displayed on a spatial light modulator (SLM), a technique called holographic femtosecond laser processing. The holographic method has the advantages such as high throughput, high light use efficiency, and variable, instantaneous, and 3D patterning. Furthermore, the use of an SLM gives an ability to correct unknown imperfections of the optical system and inhomogeneity in a sample using in-system optimization of the CGH. Furthermore, the CGH can adaptively compensate in response to dynamic unpredictable mechanical movements, air and liquid disturbances, a shape variation and deformation of the target sample, as well as adaptive wavefront control for environmental changes. Therefore, it is a powerful tool for the fabrication of biological cells and tissues, because they have free form, variable, and deformable structures. In this paper, we present the principle and the experimental setup of holographic femtosecond laser

processing, and the effective way for processing the biological sample. We demonstrate the femtosecond laser processing of biological materials and the processing properties.

10076-45, Session 10

A versatile DVD-based time-stretch imaging cytometry platform

Anson H. L. Tang, Kenneth K. Y. Wong, Kevin K. Tsia, The Univ. of Hong Kong (Hong Kong, China)

High-throughput time-stretch imaging is proven predominantly in format of microfluidic suspension, and thus in the applications of imaging flow cytometry. Nevertheless, this ultrafast technology has yet been compatible with imaging cells on solid-substrate platforms – a widespread strategy adopted in standard microscope-based imaging cytometry or cell assays. The throughput of these common image-based methods is primarily limited by the compromise between static field-of-view (FOV) and imaging speed (fps) in standard imaging sensors. Alternative methods such as automated microscope systems incorporating mechanical scanning devices can image with a large FOV without sacrificing image resolution. However, the throughput is still inherently bottlenecked by mechanical inertia. We here report a new type of time-stretch imaging cytometry platform compatible with both cells (adherent or specifically captured) and tissue sections fixed on a solid spinning substrate at known radial position and rotational speed. We implement this assay by modifying a commercial digital versatile disc (DVD) drive such that the rotational speed can be arbitrarily and stably controlled. Human breast cancer cells (MCF-7) and cartilage tissue sections are fixed on polycarbonate substrates, which are the commercial DVD, and imaged under high spinning speed (2400 rpm). The system, integrated with time-stretch imaging, can provide cellular resolution (~2 μm in radial direction) at high line-scan rate (11 MHz) and wide arbitrary spinning rate (900-4000 rpm). The integration of time-stretch imaging with this versatile DVD platform opens a wider scope of applications covering real-time monitoring of cultured living adherent cells, single-cell imaging immunoassay, ultralarge-scale tissue section imaging for digital histopathology.

10076-46, Session 10

Replacing the Fourier transformation in optical coherence tomography with multiple comparison operations

Adrian Bradu, Univ. of Kent (United Kingdom); Sylvain Rivet, Univ. of Brest (France); Adrian Podoleanu, Univ. of Kent (United Kingdom)

The conventional spectral domain (SD) and Fourier domain (FD) OCT method deliver a 1D reflectivity profile in the sample investigated by applying a Fourier transform (FT) to the channeled spectrum, CS, at the interferometer output. We discuss here the advantages of a novel OCT technology, Master Slave (MS). The MS technique delivers a single reflectivity point from a selected depth, by comparing the CS with a replica of the CS (mask). To compete with the conventional FT based technology, P replicas (masks) of the CS are prepared for P optical path difference (OPD) values using a mirror. Comparing the CS obtained with the sample in place, with the P masks, employing a comparator for each mask, P signals along P separate wires are produced, for the P points of the A-scan, all signals delivered in parallel. The MS method radically changes the main building blocks of a SD (FD)-OCT set-up. The serially provided signal in conventional technology is replaced by multiple signals, a signal for each OPD point along a line in depth in the object investigated. Three immediate advantages of the procedure will be discussed:(1) direct access to information from selected depths; (ii) elimination of the process of resampling, required by the FT based conventional technology, with immediate consequences in improving the decay of sensitivity with depth, achieving the expected axial resolution limit, reduction in the time to display an image and lower cost OCT assembly; (iii) tolerance to dispersion in the OCT interferometer.

10076-47, Session 10

Hyperspectral single-pixel imaging with dual optical combs

Kyuki Shibuya, The Univ. of Tokushima (Japan) and Japan Science and Technology Agency (Japan); Takeo Minamikawa, The Univ. of Tokushima (Japan) and Japan Science and Technology Agency (Japan); Yasuhiro Mizutani, Osaka Univ. (Japan) and Japan Science and Technology Agency (Japan); Takeshi Yasui, The Univ. of Tokushima (Japan) and Japan Science and Technology Agency (Japan); Tetsuo Iwata, The Univ. of Tokushima (Japan) and Japan Science and Technology Agency (Japan)

?Dual comb spectroscopy (DCS) is based on the combination of Fourier transform spectroscopy with an optical frequency comb (OFC), and has a spectral resolution of MHz order over a spectral range of several tens THz. Furthermore, non-mechanical optical pass length scanning enables the rapid data acquisition. However, in order to expand DCS into spectral imaging, a CCD or a CMOS camera can not be used because a high-speed, point detector is indispensable to acquire the fast interferogram signal in DCS. Therefore, the first demonstration of DCS imaging was based on the mechanical scanning of the sample position. If DCS imaging can be achieved without the need for mechanical scanning, the application field of the DCS imaging will be largely expanded.

?One promising method to achieve the scan-less 2D imaging is a single-pixel imaging (SPI). SPI enables scan-less 2D imaging by use of pattern illumination on the sample and a point detector. Also, the accumulation averaging effect in the random pattern illumination increases a signal-to-noise ratio.

?In this paper, we present combination of DCS with SPI, namely a scan-less DCS imaging. Spectral imaging of a sample with spectral dispersion indicated the effectiveness and potential of scan-less DCS imaging.

10077-1, Session 1

On-chip near field fluorescence excitation and detection with nanophotonic waveguides for enhanced surface sensitivity

Md. Mahmud-Ul-Hasan, IMEC (Belgium) and KU Leuven (Belgium); Pieter Neutens, IMEC (Belgium); Liesbet Lagae, Pol Van Dorpe, IMEC (Belgium) and KU Leuven (Belgium)

Fluorescence is a widely used transduction mechanism in bio-imaging, sensing or physical chemistry characterization applications. The ability to selectively excite desired molecules without generating considerable bulk background from nearby molecules is very important for all these applications. A near field excitation using an exponentially decaying evanescent field is often used to reduce the bulk background by selectively exciting molecules near to the surface. We propose an on-chip platform to improve the surface and bulk fluorescence separation by combining near-field excitation and near-field collection. We used the exponentially decaying evanescent tail of a Silicon Nitride rib waveguide to excite molecules and coupled the subsequent emission back via the same waveguide. We observe from the finite difference time domain simulation that both the excitation and coupling efficiency depend exponentially on the surface-molecule distance. Thus, combination of near field excitation and collection improves surface-bulk separation. A reduction by half in effective 1/e decay length was found experimentally for this combined near-field excitation and collection technique compare to the conventional only near-field excitation based technique .

An analytical model is derived to find the optimum device efficiency for bio-sensing applications and established a general condition for sensor length to maximize the device efficiency and validated by experimental data.

Finally, we used this platform for Fluorescence Correlation Spectroscopy and steady-state fluorescence anisotropy measurement.

In this talk, I will present the fabrication, characterization and experimental results obtained using this proposed waveguide based platform.

10077-2, Session 1

Full scattering profile for detecting physiological tissue properties

Hamootal Duadi, Dror Fixler, Bar-Ilan Univ. (Israel)

Light reflectance and transmission from soft tissue has been utilized in noninvasive clinical measurement devices such as the photoplethysmograph (PPG) and reflectance pulse oximeter. Most methods of near infrared (NIR) spectroscopy focus on the volume reflectance from a semi-infinite sample, while very few measure transmission.

We will show that examining the full scattering profile (FSP), which is the angular distribution of exiting photons, provides more comprehensive information when measuring from a cylindrical tissue, such as earlobe, fingertip and pinched tissue.

In our talk a Monte Carlo simulation will present an isobaric point, which is not dependent on changes in the reduced scattering coefficient. The angle corresponding to this isobaric point linearly depends on the tissue diameter. We will present the role of multiple scattering and absorption on the FSP. First we will define the range in which multiple scattering occurs for different tissue diameters. Next we will demonstrate that absorption linearly influences each intensity point of the FSP and, more importantly, the absorption does not change the position of the isobaric point.

At the end of our talk we will propose a new method to assess the oxygen saturation in venous blood, SvO2, which is related to the tissue oxygen utilization. We propose using two NIR wavelengths and collecting the backscattered light from two photo-detectors located in different distances from the light-source.

The findings of this work demonstrate a realistic model for optical tissue measurements such as NIR spectroscopy, PPG and pulse oximetery.

10077-3, Session 1

Plasmoelectronic sensor for real-time on-chip wavelength selective biosensing

Alec Cheney, Borui Chen, Tim Thomay, Alexander N. Cartwright, Univ. at Buffalo (United States)

We have developed a simple grating-based plasmonic biosensor that features an optoelectronic behavior that is enhanced by plasmonic losses. Our device may open avenues to real-time, on-chip biosensing by leveraging plasmon-induced changes in conductivity of metal nanostructures to develop a sensor that is easily integrated with existing CMOS technology. In our system, optically-induced surface plasmons interact with conduction electrons in the metal structures, resulting in an increase in electron scattering. Thus, incident light induces a detectable change in resistance (1). Our ultrafast measurements show that these scattering processes occur on extremely short timescales, indicating that these detectors may prove useful in monitoring fast bioprocesses. As seen both in our simulations and measurements, the wavelength response of our device is strongly dependent on the physical dimensions of the metal grating, with a maximum bandwidth of 30 nm. This enables a tailored response across the visible and IR spectral regions for on-chip differentiation of a wide range of fluorescent biomarkers. As a result, an array of such structures may offer the potential for fluorescence detection and imaging without the need for costly filters and optical equipment. Additionally, because the these devices use noble metals and inert dielectric materials, these devices are highly biocompatible. Consequently, these sensors may prove useful in areas from flow cytometry and fluorescence microscopy to more traditional real-time molecular detection by surface plasmon resonance (SPR).

(1) J. Kim and J. Yeo. Nano Lett. 15 (4) 2291 (2015) DOI: 10.1021/nl5043402

10077-4, Session 1

Towards biological ion imaging in vivo: potassium selective photoacoustic nanosensor

Chang H. Lee, Jeffrey Folz, Wuliang Zhang, Janggun Jo, Xueding Wang, Raoul Kopelman, Univ. of Michigan (United States)

Ion selective optical nanosensors allow highly accurate ion measurements in biological systems, without the physical limitations imposed by the use of ion selective electrodes. Optically based nanosensors, such as Photonic Explorers for Bioanalysis with Biologically Localized Embedding (PEBBLEs), have been optimized for fluorescence microscopy imaging and applied for imaging numerous biochemical analytes. In here, we report the first example of a potassium selective nanosensor optimized for photoacoustic (PA) imaging. The latter is one of the fastest emerging imaging modalities; it combines traditional ultrasound imaging and optical imaging so as to overcome the severe light penetration depth problem faced by fluorescence imaging when applied in vivo, and thus being ideal for in vivo chemical imaging. The new potassium selective nanosensor (K+-PEBBLE) shows excellent response in the biological range, from 0 to 200 mM, as confirmed

by both UV-Vis Spectroscopy and PA Spectroscopy. Furthermore, the K+-PEBBLE showed a 2 orders of magnitude, or higher, selectivity to K+, relative to any other cations, such as Li +, Na +, Ca 2+, and Mg 2+. Notably, this example of a biological ion selective PA sensor can be easily applied to other biologically relevant cations and anions based on the standard principles of ion selective optodes, and thus to their utilization for in vivo biological ion imaging. This PA K+-PEBBLE could become a valuable tool for providing real time, in-vivo, chemical imaging of biological processes related to the activity of the CNS and heart, answering questions that are not accessible by conventional methods.

10077-5, Session 1

Frequency swept laser based enhanced optical sensor using a plasmonic nanostructure

Seunghun Lee, Heesang Ahn, Tae Young Kang, Kyujung Kim, Pusan National Univ. (Korea, Republic of)

A plasmonic based sensing techniques have shown tremendous potential especially in a single molecule detection. Here, we concern a wavelength-swept laser based optical sensing techniques in a plasmonic nanostructure. We have found the sharp peaks in transmission in certain range from 1270 to 1350 nm wavelength. The shift from the change of refractive index is observed depending on the wavelength. The degree of the peak shift depends on the size of the nano-hole pattern and properties of period and the gold film thickness. We report numerical studies of the proposed two-dimensional nano-hole array to elucidate the optimum structure.

10077-6, Session 1

The trapezoidal nanostructure based surface plasmon resonance

Tae Young Kang, Hyerin Song, Kyujung Kim, Pusan National Univ. (Korea, Republic of)

The surface plasmon resonance energy emitted by bare metallic film is not enough to excite target analyt because of the small energy. Thus, the metal nanostructures on the film (such as nano-wire, nano-post) have been used to improve the low plasmon energy by localizing effect of antenna. In nanostructure, the plasmon field is highly localized at the sharpened edge of the structure because the propagating energy of surface Plasmon wave is bumped to dielectric material. Perhaps, there is another conditions enhancing Plasmon field, such as a gap of two metal, structural transformations, highly localized field can be formed. By adopting reversed trapezoidal structure, we guessed that controlling area and enhanced plasmon field from both the nano-antenna effect can be possible. So, we calculated trapezoidal nano-wire structure at many cases of different the ratio of b?ottom length and top length of nanostructure. Then we can examine the variation of Plasmon field area and intensity. Furthermore, we can acquire unusual result that the intensity of Plasmon field can have different tendencies depending on ratio and incident angle. All the simulation in this paper is calculated by Finite Element Method (FEM).

10077-52, Session 1

Quantum dots pushing up in vitro diagnostics limits

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Biopsies are conventionally performed in two dimensions. Cell or tissue samples´ thickness is not taken into account. Histological slices in general have thicknesses between 3 and 8 micrometers. Considering samples as bidimensional (thickness z=0μm) can imply that some molecular domains containing important biomarkers are nor represented in the resulting images. Besides this, the need of using agents, such as formaldehyde, may induce changes in important features of the samples, such as autofluorescence noise, changes in the permeability, etc. The use of quantum dots as fluorescent probes, enable the labelling of fresh cell and histological samples, allowing the the investigation of biomarkers' expression along the three dimensions, leading to more accurate analysis. Further, this simple methodology also allows real time monitoring of cell/tissue events at a molecular level.

Present work shows and discusses pattern and fluorescence intensity emission at the visible region obtained as a function of tissue thickness in histological (z=8.4μm) breast cancer samples labeled with fluorescent colloidaly prepared water soluble quantum dots. Series of 256 three-dimensional (3D) images were recorded from each tissue sample, under 488 nm excitation, by laser scanning confocal microscopy. In order to compare the results obtained, all the acquisition parameters were mantained constant. Results point to the possibility of significantly more accurate histological diagnostics. They clearly show distinct labeling patterns across sample thickness, as well as the presence of intratissular biomarker domains.

10077-42, Session PMon

IOT: Internet of the toilet

Yaara Kapp-Barnea, OutSense Ltd. (Israel)

Occult blood test is the simplest method of screening for the presence of bleeding polyps, indicating colorectal cancer. 80 million Americans over the age of 50 are advised to perform yearly occult blood testing. Current test kits require stool handling, leading to low compliance and suffer inherently from low sensitivity. OutSense has developed method and instrument containing sensor, illumination and energy source, a processor and a UWB broadcasting device, for automatic, continuous screening of occult blood in excretions. The method of screening is based on optically acquired information, which can point to the existence of pathology. This, by spectral and spatial image analysis of continuously, taking hundreds of frames and scan in real-time the optical signature of haemoglobin in excretions disposed within a toilet bowl.

10077-43, Session PMon

DNA molecule as a spin system and the symmetric top model

Subhamoy Singha Roy, JIS College of Engineering (India)

The relevant path integral represents a charges particle in the field of a nonquantized monopole which suggests that angular momentum is not quantized . However in a spin chain the non quantizd monopole charge appears in the renormalization group flow and corresponds to the Barry phase required by a spin ? state in an entangled spin system. Thus avoids the RLC model crisis.

10077-44, Session PMon

Full scattering profile of circular optical phantoms mimicking biological tissue

Dror Fixler, Bar-Ilan Univ. (Israel); Malgorzata

Jedrzejewska-Szczerska, Gdansk Univ. of Technology (Poland); Idit Feder, Bar-Ilan Univ (Israel)

Human tissue is one of the most complex optical media since it is turbid and nonhomogeneous. In our poster, we suggest a new type of skin phantom and an optical method for sensing physiological tissue condition, basing on the collection of the ejected light at all exit angles, to receive the full scattering profile. We have simulated light propagation in homogenous and heterogeneous cylindrical tissues and we have obtained full scattering profile. Conducted experiments were carried out on an unique set-up for noninvasive encircled measurement. Set-up consisted of a laser, a photodetector and new tissues-like phantoms made with a polyvinyl chloride-plastisol (PVCP), silicone elastomer polydimethylsiloxane (PDMS) and PDMS with glycerol mixture. Phantoms present different reduced scattering coefficients with values of about 3.5 mm-1 at 500 nm and 2.5 mm-1 at 1100 nm for PDMS with glycerol mixture phantoms and about 5 mm-1 at 500 nm and 1 mm-1 at 1100 nm PVCP. Our method reveals an isobaric point, which is independent of the optical properties. Furthermore, we present the angular distribution of cylindrical phantoms, in order to sense physiological tissue state. These findings can be useful for biomedical applications such as non-invasive and simple diagnostic of the fingertip joint, ear lobe and pinched tissues.

10077-45, Session PMon

Localized surface plasmon resonance biosensor using gold-silver alloy

Heesang Ahn, Kyujung Kim, Hyerin Song, Pusan National Univ. (Korea, Republic of)

Silver is well known for providing the sharpest SPR peak among many kinds of metals for surface plasmon resonance (SPR) sensor and has enhanced sensitivity to refractive index variation of the sample. For that reason, silver has been widely used for sensitive measurement of surface plasmon resonance (SPR) sensor. However, gold is used instead of silver for the biosensor as gold shows stable optical and chemical properties and has high biocompatibility. Nevertheless, still the sensitivity of the gold substrate is lower than of the silver substrate, so demands for high sensitivity of SPR biosensor exist. In this paper, we fabricated silver-gold alloy film to enhance the sensitivity of the SPR biosensor. Diverse proportions of the silver-gold alloy film were fabricated by evaporation of two sources at the same time. The proportions of the components, gold and silver, were monitored by EDS analysis. Then, SPR peak was practically measured on those silver-gold alloy films to confirm the effectiveness, and eventually find out the optimum ratio of gold and silver. Finally, biocompatibility tests were conducted using cell culture and cell counting analysis.

10077-46, Session PMon

Plasmonic super-localization using nano-post arrays for biomedical spectroscopy

Hongki Lee, Donghyun Kim, Yonsei Univ. (Korea, Republic of)

Plasmonic nanostructures enable field confinement which is locally amplified within sub-diffraction limited volume. The localized near-field can be useful in many biomedical sensing and imaging applications. In this research, we present the near-field characteristics localized by plasmonic nano-post arrays for biomedical spectroscopy. Circular gold nano-post arrays were modeled on gold and chrome films fabricated on a glass substrate whose thickness was 50, 20 and 2 nm, respectively. The nano-post arrays were fabricated with an e-beam lithography and a diameter of the post was 250 nm with periods varied as 500, 700, and 900 nm. The field localization produced by nano-posts was induced by angled illumination with a total internal reflection fluorescence microscope objective lens and measured by a near-field scanning optical microscope (NSOM). The NSOM has a tapered fiber probe with a 70-nm aperture and was a continuous-wave laser whose wavelength is 532 nm as light source. Incident TM-polarized light exhibited field localization on one side of an individual gold nano-post and the intensity was shown to enhance by 7.57 and 11.76 times for two periods, 500 and 700 nm, in comparison with the intensity on the bare gold film. When the direction of light incidence ? was changed to ??, localized field was switched to the opposite edge of the circular nano-post. We performed 3D rigorous coupled-wave analysis for the field calculation and confirmed the localized field distribution at given illumination angles. We also discuss the potential applications of plasmonic field localization for analysis of biomolecules, cells, and tissues.

10077-47, Session PMon

Fused micro-knots

Moti Fridman, Shir Shahal, Bar-Ilan Univ. (Israel)

We present fusing of fiber micro-knot by a CO_2 laser beams. We demonstrate tuning of the coupling strength and tuning of the spectral resonance of the micro-knot by the fusing process. The experimental results reveal that fusing the fiber micro-knots increases the coupling efficiency, and improves the robustness and the stability of the micro-knots.

10077-48, Session PMon

Investigation of nanodiamond interactions with the blood of small animal pets

Michal Wasowicz, Warsaw Univ. of Life Sciences SGGW (Poland)

Unique properties of nanodiamonds, including their mechanical and optical properties, as well as modifiable surfaces, make them an interesting novel material. They are generally used in biomedical applications, such as biomedical imaging and drug delivery. However, toxicity of carbon nanomaterials varies with their purity, size and surface functional groups. Recent studies suggest that nanodiamonds, in general, are more biocompatible than other carbon materials. Therefore, there is a need to investigate their compatibility with biological materials.

We present the optoelectronic investigation of the interaction of nanodiamond biomarkers with blood of small animal pets. In vitro interactions of blood of small animal pets and its components with different nanodiamond biomarkers have been examined. Plasmo-chemical modifications of detonation nanodiamond particles gives the possibility for controlling their surface for biological applications. Optical investigations reveal the biological activity of nanodiamonds in blood dependent on its surface properties. We compare different types of nanodiamonds: commercial non-modified detonation nanodiamonds (grain sizes from 2 to 5 nm), nanodiamonds modified by MW PACVD method, and chemically modified nanodiamonds. Further studies with optical microscopy provide insight into red blood cells morphology and its changes upon interaction with nanodiamonds. Furthermore, the influence of the nanodiamonds on white blood cells was observed, as well.

The optical microscope investigations were conducted. The appropriate amount of the patients were selected in order to obtain statistically significant results. Tests were performed for different concentrations of nanodiaments. The study was performed for different periods of time in minutes: 5, 15, 60, 180, 300.

10077-49, Session PMon

Surface-enhanced Raman spectroscopy for detection of drugs in whole human blood

Maciej S. Wróbel, Gdansk Univ. of Technology (Poland); Soumik Siddhanta, Johns Hopkins Univ. (United States); Malgorzata Jedrzejewska-Szczerska, Janusz Marek Smulko,

Gdansk Univ. of Technology (Poland); Ishan Barman, Johns Hopkins Univ. (United States)

We present a Surface-Enhanced Raman Spectroscopy approach for detection of drugs of abuse in whole human blood. We utilize a near infrared laser with 830 nm excitation wavelength in order to reduce the influence of fluorescence on the spectra of blood. However, regular plasmon resonance peak of plasmonic nanoparticles, such as silver or gold fall in a much lower wavelength regime about 400 nm. Therefore, we have shifted the plasmon resonance of nanoparticles to match that of an excitation laser wavelength, by fabrication of a silver-core gold-shell nanoparticles. By combining the laser and plasmon resonance shift towards longer wavelengths we have achieved a great reduction in background fluorescence of blood. Great enhancement of Raman signal coming solely from drugs was achieved without any prominent lines coming from the erythrocytes. We have applied chemometric processing methods, such as Principal Component Analysis (PCA) to detect the elusive differences in the Raman bands which are specific for the investigated drugs. We have achieved good classification for the samples containing particular drugs (e.g., Butalbital, Pseudoephedrine). Furthermore, a quantitative analysis was carried out to assess the limit of detection (LOD) and limit of quantification (LOQ) using Partial Least Squares (PLS) regression method. In conclusion, our LOD and LOQ values obtained for each class of drugs was competitive with the gold standard GC/MS method.

10077-50, Session PMon

Remote optical stethoscope and optomyography sensing device

Mark Golberg, Sage Polani, Nisan Ozana, Yevgeny Beiderman, Javier Garcia, Joaquin Ruiz-Rivas Onses, Martin Sanz Sabater, Max Shatsky, ContinUse Biometrics Ltd. (Israel); Zeev Zalevsky, Bar-Ilan Univ. (Israel)

In this paper we present the usage of photonic remote device for sensing nano vibrations for detection of muscle contraction and fatigue, eye movements and in-vivo estimation of glucose concentration. The same concept is also used to realize a remote optical stethoscope. The advantage of doing the measurements from distance is in preventing passage of infections as in the case of optical stethoscope or in the capability to monitor e.g. sleep quality without disturbing the patient. The remote monitoring of glucose concentration in the blood stream and the capability to perform optomyography for the Messer muscles (chewing) is very useful for nutrition and weight control. The optical configuration for sensing the nano vibrations is based upon analyzing the statistics of the secondary speckle patterns reflected from various tissues along the body of the subjects. Experimental results present the preliminary capability of the proposed configuration for the above mentioned applications.

10077-7, Session 2

Surface modified gold nanoparticles for SERS based detection of vulnerable plaque formations *(Invited Paper)*

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Cardiovascular diseases are the leading cause of death worldwide. Atherosclerosis is closely related to the majority of these diseases, as a process of thickening and stiffening of the arterial walls through accumulation of lipids, which is a consequence of aging and life style. Atherosclerosis affects all people in some extent, but not all arterial plaques will necessarily lead to the complications, such as thrombosis, stroke and heart attack. One of the greatest challenges in the risk assessment of atherosclerotic depositions is the detection and recognition of plaques which are unstable and prone to rupture. These vulnerable plaques usually consist of a lipid core that attracts macrophages, a type of white blood cells that are responsible for the degradation of lipids. It has been hypothesized that the amount of macrophages relates to the overall plaque stability. As phagocytes, macrophages also act as recipients for nanoscale particles or structures. Administered gold nanoparticles are usually rabidly taken up by macrophages residing within arterial walls and can therefore be indirectly detected. A very sensitive strategy for probing gold nanoparticles is by utilizing surface enhanced Raman scattering (SERS). By modifying the surface of these particles with SERS active labels it is possible to generate highly specific signals that exhibit sensitivity comparable to fluorescence. SERS labeled gold nanoparticles have been synthesized and the uptake dynamics and efficiency on macrophages in cell cultures was investigated using Raman microscopic imaging. The results clearly show that nanoparticles are taken up by macrophages and support the potential of SERS spectroscopy for the detection of vulnerable plaques.

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10077-8, Session 2

Blood pulse wave velocity and pressure sensing via fiber based and free space based optical sensors *(Invited Paper)*

Talia Sirkis, Yevgeny Beiderman, Sergey Agdarov, Yafim Beiderman, Zeev Zalevsky, Bar-Ilan Univ. (Israel)

Continuous noninvasive measurement of vital bio-signs, such as cardiopulmonary parameters, is an important tool in evaluation of the patient’s physiological condition and health monitoring. On the demand of new enabling technologies, some works have been done in continuous monitoring of blood pressure and pulse wave velocity. In this paper, we introduce two techniques for non-contact sensing of vital bio signs. In the first approach the optical sensor is based on single mode in-fibers Mach-Zehnder interferometer (MZI) to detect heartbeat, respiration and pulse wave velocity (PWV). The introduced interferometer is based on a new implanted scheme. It replaces the conventional MZI realized by inserting of discontinuities in the fiber to break the total internal reflection and scatter/collect light. The proposed fiber sensor was successfully incorporated into shirt to produce smart clothing. The measurements obtained from the smart clothing could be obtained in comfortable manner and there is no need to have an initial calibration or a direct contact between the sensor and the skin of the tested individual. In the second concept we show a remote noncontact blood pulse wave velocity and pressure measurement based on tracking the temporal changes of reflected secondary speckle patterns produced in human skin when illuminated by a laser beams. In both concept experimental validation of the proposed schemes is shown and analyzed.

10077-9, Session 2

Biocomputation with nanoscale biological agents

Dan V. Nicolau, McGill Univ. (Canada)

No Abstract Available

10077-10, Session 2

Nanostructured hybrid cavity-coupled plasmonic biosensor for dopamine detection

Abraham Vázquez-Guardado, Swetha Barkam, Soumen Das, Sudipta Seal, Debashis Chanda, Univ. of Central Florida (United States)

Dopamine is a critical neurotransmitter in our nervous system whose abnormal levels are associated with the neuropsychiatric disorder (depression, drug and alcohol dependence, Parkinson's disease), pathogenesis of psychosis (schizophrenia) and neural cancer. Detection of dopamine with high sensitivity and specificity could help in basic understanding of the pathophysiology, drug development, and disease management. However, detection of dopamine is very challenging due to its low molecular weight. To achieve this goal we used a new class of cavity-coupled plasmonic biosensor. The low quality resonance of localized surface plasmon (LSP) can be dramatically enhanced when coupling them to high quality photonic resonances. The cavity-coupled LSP resonance wavelength is very sensitive to minute environmental fluctuations induced by the change in the local refractive index. In this work we present a hybrid plasmonic biosensor functionalized with engineered oxygen-deficient cerium oxide nanoparticle (CNPs). CNPs selectively form a complex with dopamine due to its unique redox activity at nanoscale. When colloidal CNPs absorb dopamine its optical properties change, which is observed as a spectral shift of the scattering cross section. When CNPs reside on the LSP sensor, such complex formation is transferred to the LSP resonances experiencing a spectral shift. We demonstrate femtomolar concentration of dopamine detection in simulated body fluid using the hybrid LSP sensor.

10077-500, Session Plen

Porous silicon nanoparticles as self-reporting drug delivery vehicles

Michael J. Sailor, Univ. of California, San Diego (United States)

There is increasing emphasis on biomedical devices that incorporate graceful or sudden degradation into their designs. For in vivo applications, the material components and their degradation products must also be non-toxic. Although bulk silicon is too stable to exhibit significant degradation in the body, nanoscale silicon is readily degradable and quite biocompatible. This presentation will discuss the chemistry and photochemistry of luminescent porous silicon, with emphasis on the self-destruction and reconstruction processes that can be harnessed for various in vitro and in vivo imaging and drug delivery tasks.

10077-11, Session 3

Low-coherence sensors with nanolayers for biomedical sensing

Mateusz Ficek, Marzena Hirsch, Daria Majchrowicz, Katarzyna Karpienko, Julia Milewska, Bartlomiej Dec, Pawel Wierzba, Gdansk Univ. of Technology (Poland); Przemyslaw Struk, Silesian Univ. of Technology (Poland); Mikhael Bechelany, Univ. Montpellier (France); Malgorzata Jedrzejewska-Szczerska, Gdansk Univ. of Technology (Poland)

In the last decade low-coherence sensors have gained popularity as a biophotonic sensing.

This has been possible because of the application of the new materials in fiber optic technology. The nanolayers made from the materials such as: nanodiamond (NCD), boron-doped nanodiamond (B-NCD), zinc oxide (ZnO), titanium dioxide (TiO2), aluminum oxide (Al2O3) and boron nitride (BN) have been applied in the construction of low-coherence sensors in fiber optic configuration. Nanodiamond and boron-doped nanodiamond have been made by the Chemical Vapor Deposition (CVD), hence metallic based layers were deposited in the Atomic Layer Deposition Process. They have been used as a protective coating, reflective layers and as a sensing medium. Sensors have been made with the use of standard telecommunication single-mode optical fiber (SMF-28). The thickness of the layers were used in the experiments were in the range of 30 nm to 200 nm. Nanodiamond and boron-doped nanodiamond layers have been made by the Chemical Vapor Deposition (CVD), hence metallic based layers were deposited in the Atomic Layer Deposition Process. Measurements were performed with broadband sources operating at 930 nm, 1300 nm and 1550 nm. Interference signal was acquired by an optical spectrum analyzer. Measured signal were analysed in the spectrum domain. Any change of the phase difference between interfering beams reflected from the sensor head depends on measurand occured in the spectrum of the measurement signal.

The fiber optic low-coherence sensors using nanolayers and their ability to perform biophotonic measurements will be presented.

10077-12, Session 3

Improved borders detection of areas enriched with gold nanoparticles inside biological phantom

Zeev Zalevsky, Bar-Ilan Univ. (Israel); Yossef Danan, Bar-Ilan Univ (Israel); Moshe Sinvani, Bar-Ilan Univ. (Israel)

The ability to resect tumor completely is a key merit in preventing recurrence of the disease. In order to achieve more complete tumor resection the surgeon must to clearly identify the tumor margins. This identification is even more crucial when the tumor growth adjacent or in neurological structure, therefore it is impossible to remove extra tissue. The method proposed in this paper is a photothermal imaging using temporally flickering targeted gold nanoparticles (GNP) and a thermal camera. By illuminating the GNPs with a wavelength corresponded to their localized surface plasmonic resonance the GNPs absorb optical energy which turns to heat of the GNPs and their environment. Those particles are specifically target and decorate the surface of the cancer cells. Thus, the temperature elevation occurs adjacent to the cancerous tissue enabling us to distinguish between cancerous and noncancerous cells by using the thermal camera. In order to maximize the distinction of the tumor margins a modulation of the laser was taken to minimalized heat dissipation to noncancerous tissue by conductive and convective heat transfer. Using photothermal imaging instead of the widely used cell imaging applications based on light-scattering modalities allow us to overcome the background noise caused by light scattering from the tissue, thus improving the SNR and having higher contrast between the target cancerous cells and the healthy tissue.

10077-13, Session 3

Models and Raman analysis of molecular nanofilms conjugated on 2D photonic crystal slabs

Zheng Wang, Chao Liu, The Univ. of Texas at Austin (United States); Erwen Li, Oregon State Univ. (United States); Swapnajit Chakravarty, Omega Optics, Inc. (United States); Alan X. Wang, Oregon State Univ. (United States); D. L. Fan, The Univ. of Texas at Austin (United States); Ray T. Chen, The Univ. of Texas at Austin (United States) and Omega Optics, Inc. (United States)

Two dimensional (2D) photonic crystal slabs (PCS), which generally consist of arrays of air holes in the top layer of a dual layer dielectric film, have been demonstrated as a promising platform for optical biosensing. Both the Fano resonance in perfect 2D PCS and the Lorentzian resonance in PC micro-cavity resulted from introduced defects in 2D PCS has been studied. While, the use of resonance peak shift for detecting molecules owing to the change of the refractive index is a nonspecific biosensing technique. Biorecognition molecules, such as antibodies that can specific bond to interesting molecules, are conjugated on the 2D PCS to improve the detection specificity. It is a widely adopted assumption that the conjugated molecules form into a uniform nanofilm in the 2D PCS based biosensors, which covers either the entire surface of the dielectric layer or the entire sidewalls of air holes. However, the actual device performance is much lower than that obtained based on this assumption, which suggests the over-simplicity of the aforementioned assumption. It is of great interest to reveal the actual arrangement and distribution of molecules on 2D PCS for designing high performance 2D PCS biosensors. Here, we propose models and analysis of the distribution of nanofilms on 2D PCS. We employed Raman scattering technique to experimentally reveal the actual various configurations of nanofilms, which support our theoretical modeling excellently. The results obtained in this research can be essential for designing high performance PCS based nano-biosensors.

10077-14, Session 3

Plasmonic sensor for troponin I detection using whole blood

Xu Han, Hossein Shokri Kojori, Roger M. Leblanc, Sung Jin Kim, Univ. of Miami (United States)

Surface Plasmon Resonance (SPR) has been widely studied for various application. Due to the highly sensitive optical property to the change of the refractive index of the surrounded medium, there have been lots of reports for biological sensing. Direct Plasmon-to-Electric conversion device using metal nanostructures and semiconductor does not require additional readout optics and the device size and sensing area could be much smaller (1/100 to 1/10 of size) than current technologies. In addition, our sensing platform designed to address the issue of using the colored medium (e.g. whole blood) for detection. The detection signal comes only from plasmonic absorption and is not affected by the absorption from the medium. We developed a plasmonic sensing platform using a metal-semiconductor-metal detector by incorporating gold nanostructures on top of the semiconducting layer. The gold nanostructures are functionalized using antibodies to detect Troponin I, which is very important molecule to prevent hart attacks. In this presentation, we report a successful demonstration of a point-of-care sensing platform to detect cardiac Troponin I using antibody functionalized plasmonic nanostructures. Because the sensors are integrated into a microfluidic channel, it requires only a few μl of sample volume. The limit of detection was 20 pg/ml in our preliminary results, and we successfully demonstrated sensor operation using whole blood. This plasmonic sensor has several advantages such as extremely small size for the point-of-care system, multiplexing capability, no need of complex optical geometry and real-time binding monitoring.

10077-16, Session 3

Glucose-functionalized gold nanoparticles as a metabolically targeted CT contrast agent for distinguishing tumors from non-malignant metabolically active processes *(Invited Paper)*

Tamar Dreifuss, Menachem Motiei, Oshra Betzer, Bar-Ilan Univ. (Israel); Aron Popovtzer, Rabin Medical Ctr., Beilinson Campus (Israel); Galith Abourbeh, Eyal Mishani, Hadassah-Hebrew Univ. Hospital (Israel); Rachela Popovtzer, Bar-Ilan Univ. (Israel)

In the present study, we demonstrate a novel nanoparticle- based CT imaging methodology that overcomes the main drawbacks of the currently used [18F]FDG-PET: (1) 2GF-GNP is cancer-specific and allows the distinction between cancer and inflammatory processes, (2) it offers cancer detection and imaging with no dependence on short-lived radio-tracers, and (3) it provides simultaneous anatomical and functional information using CT. In addition, unlike specific immune- targeting approaches, this imaging modality does not target the expression of one molecule, but provides unique data about the functional state of the tumor tissue.

We further showed that despite the conjugation to the GNP, the glucose molecule preserves some of its activity, allowing glucose recognition and cellular internalization, probably by receptor mediated endocytosis. In addition, we showed that due to the unique characteristics of tumor vasculatures and dissimilarities between cancer and inflammatory processes, accumulation of GNPs occurs in the tumor and not in the inflammatory lesion, thus preventing false-positive results. Future research should be done to optimize the system, such as optimization of GNPs' size which may play an important role in view of the EPR effect.

Finally, our new concept of functional CT imaging provides a new set of capabilities in cancer detection, staging and follow- up, and can be applicable to a wide range of cancers that exhibit high metabolic profiles.

10077-17, Session 3

Plasmonic nanostars as signal enhancers for optical imaging and surface-enhanced vibrational spectroscopy (SEVS)

Olga Bibikova, Univ. of Oulu (Finland) and art photonics GmbH (Germany) and Univ. Ulm (Germany); Julian Haas, Ángela I. López-Lorente, Univ. Ulm (Germany); Alexey P. Popov, Alexander Bykov, Univ. of Oulu (Finland) and ITMO Univ. (Russian Federation); Matti Kinnunen, Univ. of Oulu (Finland); Valery V. Tuchin, N.G. Chernyshevsky Saratov National Research State Univ. (Russian Federation) and National Research Tomsk State Univ. (Russian Federation) and Institute of Precision Mechanics and Control RAS (Russian Federation); Igor Meglinski, Univ. of Oulu (Finland) and Interdisciplinary Lab. of Biophotonics, National Research Tomsk State Univ. (Russian Federation) and ITMO Univ. (Russian Federation); Boris Mizaikoff, Univ. Ulm (Germany)

Plasmonic gold nanostars (NSts) demonstrate an enhanced electric field in their surrounding due to large number of 'hot spots' on their surface resulting in a unique ability to confine light within a nanometric volume. We are demonstrating beneficial properties of NSts as signal enhancers for tissue and cell imaging using optical coherence tomography (OCT), microscopy, surface-enhanced vibration spectroscopy (SEVS), including surface-enhanced Raman scattering (SERS), and surface-enhanced

infrared absorption spectroscopy (SEIRAS) with an attenuated total reflectance (ATR) and infrared reflection-absorption spectroscopy (IRRAS) configurations.

Scattering ability of gold NSts with various sizes was investigated by OCT capillary imaging and light and confocal microscopy in vitro. The variation of NSts sizes allows one to shift plasmon resonance up to 1300 nm. The most intensive scattering signals were found from the largest NSts.

NSts were applied in SEVS scenarios using plasmonic chip-based systems containing self-assembled NSts on a silicon substrate both by evaporation and subsequent immobilization mediated by a gold layer and modified-dimercapto polyethylene glycol. The plasmonic substrates are able to concomitantly enhance Raman and mid-infrared signals. SERS and SEIRAS properties of such substrates were demonstrated. For SERS, by using crystal violet as a model analyte. The IR absorbance of analyte molecules placed on NSt-film deposited on a Si ATR crystal was up to 10 times higher for thioglycolic acid and 2 times higher for bovine serum albumin compared to a bare Si waveguide. For the best of our knowledge, this is the first attempt to use NSt-based substrate for SEIRAS studies.

10077-19, Session 3

Label-free biosensor using a light-emitting nano structures

SunYoung Kim, Seokho Kim, Jinho Choi, Hyeong Tae Kim, Ho Jin Lee, Dong Hyuk Park, Inha Univ. (Korea, Republic of); Sunjong Lee, KITECH (Korea, Republic of)

We reported on the protein sensing using a light-emitting nano structures (NSs). The aptamer were easily attached to the light-emitting NSs through electrostatic interaction between the negative counter-ions and the terminal amine (NH3+) group attached at the end of the aptamer. After the functionalization aptamer and their label–free recognition of target protein onto the surface of light-emitting NSs, the light-emitting color and intensity of a light-emitting NS were dramatically changed due to the conformational changes of the protein main chains and fluorescence resonance energy transfer. We observed color change of a light-emitting NS after attaching the aptamer, and then luminescence intensity of a single light-emitting NS was dramatically enhanced by hybridizing protein. The conformational changes of the light-emitting NS main chains due to attaching aptamer were investigated ultraviolet-visible absorption and confocal Raman spectra. The enhanced PL of the aptamer/protein can be explained in terms of the dopant-mediated fluorescence chain reaction.

10077-20, Session 3

980 nm and 808 nm excitable upconversion nanoparticles for the detection of enzyme related reactions

Sandy F. Himmelstoß, Lisa M. Wiesholler, Markus Buchner, Verena Muhr, Susanne Märkl, Antje J. Baeumner, Thomas Hirsch, Univ. Regensburg (Germany)

Upconverting luminescent nanoparticles (UCNPs) represent a unique class of nanomaterials. Due to their excitation in the near infrared region of the spectra, no fluorescence of biological compounds is trigged. Compared to other nanomaterials like quantum dots they exhibit low cytotoxicity, high photostability, no blinking and chemical inertness. These properties make them ideal for the online detection of L-lactate, an important biomarker for critically ill patients.

Here we report on UCNPs which were used for the sensing of L-lactate via an enzymatic related reaction. For this purpose, the particles were designed so that their optical properties overlap with those of the co-enzyme NADH. Under near-infrared cw laser excitation, UCNPs with an Yb3+/Tm3+ doped NaYF4 host lattice exhibit two emission bands peaking at 340 and 450 nm. The one at shorter wavelength nicely overlaps with the absorption of NADH between 340 and 360 nm. This match can be used to design enzymatic biosensors on nanoscale dimensions. Therefore, the UCNPs and L-lactate hydrogenase (which uses NAD+ as co-enzyme) were entrapped in a thin polyacrylamide hydrogel and assembled in a miniaturized flow cell. After injecting a sample containing the analyte L-lactate and NAD+, the enzyme converses L-lactate selectively to pyruvate. The reduction of NAD+ leads to NADH which affects the emission intensity at 340 nm. The luminescence intensity at 450 nm is not changed and deals as an internal reference. The sensor operates fully reversible and due to the colloidal stability, these particles can also be used as probes in imaging applications.

10077-21, Session 3

Sensing probability enhancement based on folded subwavelength grating waveguide ring resonator

Lijun Huang, The Univ. of Texas at Austin (United States) and Beijing Univ. of Posts and Telecommunications (China); Hai Yan, The Univ. of Texas at Austin (United States); Xiaochuan Xu, Naimei Tang, Swapnajit Chakravarty, Omega Optics, Inc. (United States); Huiping Tian, Beijing Univ. of Posts and Telecommunications (China); Ray T. Chen, The Univ. of Texas at Austin (United States) and Omega Optics, Inc. (United States)

Nanophotonics based label-free bio-sensors have been attracting tremendous interest in the past decade because of their high sensitivity, low cost, small sample volume requirement, and simplicity of operation. These merits are especially important for point-of-care (PoC) applications, such as viruses, streptavidin, proteins, breast cancer biomarker and DNA, however, due to the extremely small sensing surface, the probability that the analyte interacts with the optical field drops as its concentration decreases. As a result, the detection time is prohibitively long, which is widely accepted as a critical issue for nanophotonics sensors. In order to enhance the detection probability, we designed and experimentally demonstrated a folded subwavelength grating waveguide ring resonator (FSWGRR) which consists of many straight and bend subwavelength grating (SWG) waveguides. For a subwavelength grating waveguide ring resonator (SWGRR) with 50?m radius, the width, thickness and period of the subwavelength grating waveguide of 500nm, 220nm and 200nm, respectively, the sensing surface is 566.8?m^2 by calculating the surface area of each pillar, which is much larger than the surface of the top and sidewalls of a conventional ring resonator with the same radius (295.2?m^2), whereas an FSWGRR covering a same region ~ of 50?50?m^2 can have a surface area of 1700.3?m^2. Therefore, detection probability is 3 times of SWGRR and 5.76 times of conventional ring resonators, which can be further enhanced with a narrower separation of SWG waveguides.

10077-22, Session 4

Harnessing plasmon-enhanced Raman spectroimmunoassay for multiplexed and ultrasensitive detection of serological breast cancer markers

Ishan Barman, Ming Li, Johns Hopkins Univ. (United States)

Two critical, unmet needs in breast cancer are the early detection of cancer metastasis and recurrence, and the sensitive assessment of temporal changes in tumor burden in response to therapy. The present research is directed towards developing a non-invasive, ultrasensitive and specific tool that provides a comprehensive real-time picture of the metastatic tumor burden and provides a radically new route to address these overarching challenges. As the continuing search for better diagnostic and prognostic clues has shifted away from a singular focus on primary tumor lesions,

circulating and disseminated biomarkers have surfaced as attractive candidates due to the intrinsic advantages of a non-invasive, repeatable "liquid biopsy" procedure. However, a reproducible, facile blood-based test for diagnosis and follow-up of breast cancer has yet to be incorporated into a clinical laboratory assay due to the limitations of existing assays in terms of sensitivity, extensive sample processing requirements and, importantly, multiplexing capability. Here, by architecting nano-structured probes for detection of specific molecular species, we engineer a novel plasmon-enhanced Raman spectroscopic platform that offers a paradigmatic shift from the capabilities of today's diagnostic test platforms. Specifically, quantitative single-droplet serum tests reveal ultrasensitive and multiplexed detection of three key breast cancer biomarkers, cancer antigen 15-3 (CA15-3), CA27-29 and carcinoembryonic antigen (CEA), over several order of magnitude range of biomarker concentration and clear segmentation of the sera between normal and metastatic cancer levels.

10077-23, Session 4

Measurement of nanoparticle size, suspension polydispersity, and stability using near-field optical trapping and light scattering

Perry Schein, Dakota O'Dell, David Erickson, Cornell Univ. (United States)

Nanoparticles are becoming ubiquitous in applications including diagnostic assays, drug delivery and therapeutics. However, there remain challenges in the quality control of these products. Here we present methods for the orthogonal measurement of these parameters by tracking the motion of the nanoparticle in all three special dimensions as it interacts with an optical waveguide. These simultaneous measurements from a single particle basis address some of the gaps left by current measurement technologies such as nanoparticle tracking analysis, ?-potential measurements, and absorption spectroscopy. As nanoparticles suspended in a microfluidic channel interact with the evanescent field of an optical waveguide, they experience forces and resulting motion in three dimensions: along the propagation axis of the waveguide (x-direction) they are propelled by the optical forces, parallel to the plane of the waveguide and perpendicular to the optical propagation axis (y-direction) they experience an optical gradient force generated from the waveguide mode profile which confines them in a harmonic potential well, and normal to the surface of the waveguide they experience an exponential downward optical force balanced by the surface interactions that confines the particle in an asymmetric well. Building on our Nanophotonic Force Microscopy technique, in this talk we will explain how to simultaneously use the motion in the y-direction to estimate the size of the particle, the comparative velocity in the x-direction to measure the polydispersity of a particle population, and the motion in the z-direction to measure the potential energy landscape of the interaction, providing insight into the colloidal stability.

10077-24, Session 4

Impact of the light coupling on the sensing properties of photonic crystal cavity modes

Kumar Saurav, Nicolas Le Thomas, Univ. Gent (Belgium) and IMEC (Belgium)

Optimally coupling light in an integrated Photonic crystal (PhC) cavity is challenging, but crucial for improving their sensing properties. Here we experimentally investigate the impact of side coupling and in-line coupling on the transmission properties of integrated silicon PhC based air-slot cavities by probing the near field of the cavity mode with a nano fiber tip. These cavities were fabricated with standard deep UV lithography. Positioning of this nano-tip near and inside 130 nm wide Phc slot cavity modifies the dielectric map of the cavity which perturbs the intensity scattered from the cavity surface. We show that the mapping of the nano tip induced intensity variations provides more insight about the nature of the confinement of electric field of the various modes of slot cavities. Such intensity maps carry moreover information about the cavity light coupling, which is useful for maximizing the intensity of PhC slot cavity modes.

Summary:

We present a scattering near field optical technique to probe the field distribution in the near field of a PhC slot cavity by scanning a nano-tip approaches the cavity surface. We will show that how we control the positioning of a nano-tip near cavity surface and map any cavity mode. This technique allows us to provide better understanding of impact of light coupling in PhC cavity modes and can also be very sensitive in distinguishing nanoparticle of various size.

10077-26, Session 4

Extended depth of focus and aberration correction using time multiplexing

Asaf Ilovitsh, Bar-Ilan Univ. (Israel); Gilad Rand, Bar-Ilan University (Israel) and Bar-Ilan University (Israel); Shilo Levavi, Bar-Ilan University (Israel); Zeev Zalevsky, Bar-Ilan Univ. (Israel)

We propose using the time multiplexing super resolution method in order to extend the depth of focus of an imaging system. In standard time multiplexing the super resolution is achieved by generating duplication of the optical transfer function (OTF) in the spectrum domain by using moving gratings which have a basic frequency equals to the diffraction cutoff frequency. While the system is in focus, the generated OTF duplications fit perfectly, and a synthetic aperture is generated (extension of the original OTF). However, if there is a defocus error the OTF suffers from contrast reduction, and the generated duplications will result with a very unbalanced OTF which limits the depth of focus. By modifying the grating's frequency to be half of the diffraction cutoff frequency, the spectral duplications of the OTF will be closer to the original OTF position. In this case, even when the system is out of focus the defocused OTF duplications will still overlap and generate a synthetic OTF, resulting with an extend the depth of focus. In addition, the OTF of an imaging lens which has geometrical aberrations, and is not diffraction limited, also suffers from OTF contrast reduction. Thus, using the same concept, it is also possible to correct geometrical aberrations of the imaging lens. The proposed method is presented analytically, demonstrated via numerical simulations, and validated by a laboratory experiments.

10077-27, Session 4

Time multiplexing super-resolution nanoscopy based on the Brownian motion of gold nanoparticles

Tali Ilovitsh, Asaf Ilovitsh, Bar-Ilan Univ. (Israel); Omer Wagner, Bar Ilan Univ (Israel); Zeev Zalevsky, Bar-Ilan Univ. (Israel)

Super-resolution localization microscopy has revolutionized the observation of living structures at the cellular scale, by achieving a spatial resolution that is improved by more than an order of magnitude with compared to the diffraction limit. These methods localize single events from isolated sources in repeated cycles in order to achieve super-resolution. Fluorescent probes are the most commonly used contrast agents for the labeling of the biological samples, however typical drawbacks of these probes are the autofluorescence of live cells, the photo toxicity of FPs to living organisms and photobleaching. This work presents an alternative time multiplexing super-resolution technique, that does not require the labeling of the sample with contrast agents and is performed in a conventional light microscope.

Gold nanoparticles in a solution are distributed on top of the sample. The gold nanoparticles move in a random Brownian motion flow, and interact with the sample where they encode the sub-wavelength features. Due to the nanometric size of the gold nanoparticles, they are barely seen in white light illumination, therefore the illumination is performed in a wavelength that matches the surface plasmon resonance frequency of the gold nanoparticles where they become visible. A sequence of images of the sample is captured and decoded by digital post processing to create the super-resolution image. The achievable resolution is limited by the size of the gold nanoparticles.

10077-28, Session 4

Contact microspherical nanoscopy: from fundamentals to biomedical applications *(Invited Paper)*

Vasily N. Astratov, The Univ. of North Carolina at Charlotte (United States); Alexey V Maslov, N.I. Lobachevsky State Univ. of Nizhni Novgorod (Russian Federation); Aaron M. Brettin, Farzaneh Abolmaali, Yuri E. Nesmelov, The Univ. of North Carolina at Charlotte (United States); Nicholaos I. Limberopoulos, Dennis E. Walker Jr., Augustine M. Urbas, Air Force Research Lab. (United States)

There are several super-resolution mechanisms in a label-free microscopy based on nonlinear reduction of the point-spread function, near-field scanning, magnifying near-field by superlens, and informational approaches such as structured illumination. In this invited presentation, we study fundamental mechanisms of contact microspherical nanoscopy based on exact numerical solution of the Maxwell's equations. It is shown that imaging of incoherent point sources at the wavelengths resonant with the whispering gallery modes in microspheres far exceeds the classical diffraction limit in air. This mechanism is fundamentally different from the solid immersion lens concept. In contrast, visualization of the coupled modes excited in plasmonic or photonic nanostructures has an unusual property that some features of the object can be observed with practically unlimited resolution (determined by the noise), but this mechanism belongs to coherent imaging where the shape of the image can be quite different from the object. Defining the resolution in the case of biomedical applications is a more complicated task because the shape and size of the objects are less reproducible. Still, careful analysis of the images reveals some resolution advantage of contact microspherical nanoscopy over conventional microscopy. This is illustrated by super-resolution imaging of the actin protein filaments and by label-free imaging of the yeast cells.

10077-29, Session 4

Photothermal nanoparticles as molecular specificity agents in interferometric phase microscopy *(Invited Paper)*

Natan T. Shaked, Tel Aviv Univ. (Israel)

I review our latest advances in wide-field interferometric imaging of biological cells with molecular specificity, obtained by time-modulated photothermal excitation of gold nanoparticles. Heat emitted from the nanoparticles affects the measured phase signal via both the nanoparticle surrounding refractive-index and thickness changes. These nanoparticles can be bio-functionalized to bind certain biological cell components; thus, they can be used for biomedical imaging with molecular specificity, as new nanoscopy labels, and for photothermal therapy. Predicting the ideal nanoparticle parameters requires a model that computes the thermal and phase distributions around the particle, enabling more efficient phase imaging of plasmonic nanoparticles, and sparing trial and error experiments of using unsuitable nanoparticles. We thus developed a new model for predicting phase signatures from photothermal nanoparticles with arbitrary parameters. We also present a dual-modality technique based on wide-field photothermal interferometric phase imaging and simultaneous ablation to selectively deplete specific cell populations labelled by plasmonic nanoparticles. We experimentally demonstrated our ability to detect and specifically ablate in vitro cancer cells over-expressing epidermal growth factor receptors (EGFRs), labelled with plasmonic nanoparticles, in the presence of either EGFR under-expressing cancer cells or white blood cells. This demonstration established an initial model for depletion of circulating tumour cells in blood. The proposed system is able to image in wide field the label-free quantitative phase profile together with the photothermal phase profile of the sample, and provides the ability of both detection and ablation of chosen cells after their selective imaging.

10077-30, Session 4

nano-FTIR: Imaging and spectroscopy with nanometer spatial resolution

Max Eisele, neaspec GmbH (Germany)

Scattering-type scanning near-field optical microscopy (s-SNOM) has evolved as one of the key techniques to overcome the fundamental diffraction limit of conventional optical microscopy or spectroscopy. This technology enables optical measurements with a spatial resolution of 10 nanometer from the visible down to the far-infrared spectral range. s-SNOM employs a sharp metallic AFM tip that is illuminated with light to create a nanoscale optical hot-spot at the surface of a sample below the AFM probe. The local dielectric properties (refractive index) of the sample precisely determine the near-field interaction of the coupled tip-sample system. Phase and amplitude-resolved detection of the elastically back-scattered radiation as function of tip position can therefore be used to directly image the optical response of the sample on the 10-nm length scale, while simultaneously recording the AFM topography.

The development of Fourier transform infrared spectroscopy on the nanoscale (nano-FTIR) can now be used to routinely perform infrared spectroscopy of e.g. complex polymer nanostructures or even single biomolecules. In addition, embedded structural phases in biominerals (bone), organic semiconductors or functional semiconductor nanostructures can now directly be visualized and characterized on the nanometer length scale. In this presentation we will introduce the basic principle of near-field microscopy and nano-FTIR for imaging and spectroscopy with 10 nanometer spatial resolution and address their impact and key applications in the field of organic and bio-materials.

10077-31, Session 4

Electrospun silk fibroin nanofibers loaded with zataria multiflora boiss for biomedical application

Soudabeh Hajahmadi, Islamic Azad Univ. of Najafabad (Iran, Islamic Republic of)

Silk fibroin (SF) nanofiber mat is viewed as a promising base material for health care and biomedical applications. In this study, SF nanofibers loaded with Zataria multiflora Boiss, a medicinal plant well known for its traditional medical applications including its antimicrobial activity, antiseptic and analgesic, were successfully prepared utilizing an electrospinning process. The SF solutions containing (1 and 5 wt. %) ZF extract were studied for electrospinning into nanoscale fiber mats.

The morphology, porosity and conformational structures of as-spun and chemically treated SF nanofibers were investigated by wide angle X-Ray diffraction (WAXD), scanning electron microscopy (SEM), attenuated total reflectance infrared spectroscopy (ATR-IR) which demonstrated that ZF extract is likely to be present as crystalline aggregates in the nanofibers, (NMR) spectroscopy analysis showed that the chemical structure of ZF extracts was preserved during the electrospinning process.

With grate drug stability and high drug-loading efficacy, incorporation of ZM extract in the polymer media did not seem to impact the morphology of the subsequent nanofibers, as both the drug-free and the ZM-loaded composite fibers remained unchanged, microscopically.

Antibacterial activity of herbal drug incorporated SF nanofibers were determined against some gram negative and gram positive bacteria. The antibacterial effects of the ZM extract-loaded nanofiber indicated that nanofiber mat were able to inhibit the growth of the bacteria strains therefore it could act not only as a drug delivery system but also in the treatment of wound healing or dermal bacterial infections thereby proving a potential application for use as biomedical textile.

10077-32, Session 5

Off resonance long period fiber gratings for optical detection *(Invited Paper)*

Moti Fridman, Avi Klein, Shir Shahal, Gilaad Masri, Bar-Ilan Univ. (Israel)

We present long period fiber gratings with off-resonance spectral response. Our long period fiber gratings are composed of periodic structure of strong perturbations in the fiber diameter which results in unique spectral response even in off-resonance frequencies. Writing these long period fiber grating is based on utilizing the mechanical vibrations of tapered fibers during the tapering process. This writing method is simple, robust with high efficiency and high reproducibility. It also enables real-time tunability of the periodicity, efficiency and length of the grating. We also demonstrate complex grating by writing multiple gratings one on top of the other. Finally, We utilized the formation of the gratings in different fiber diameters to investigate the Young's modulus of tapered fibers.

10077-33, Session 5

Preparation and bioapplication of electrospun carbon dots-silica composite nanofibers

Zheng Xie, Chinese Academy of Sciences (China); Yong Liu, Beijing Univ. of Chemical Technology (China)

Carbon dots (CDs) are a new class of carbon nanomaterials and luminescent materials. Due to their fascinating properties, CDs can be widely applied in many fields, including bioimaging, biosensors, and photocatalysis. Performance characteristics of CDs are mainly concentrated in diluted solutions for a long time, it is possible to play advantages of small size effect and excellent cell permeability. However, the single morphology limits the development and applications cannot make progress further. To take full advantage of the low-toxicity, strong-luminescence CDs in the fields of biophotonics and optoelectronics, it is desirable to embed them in an appropriate solid matrix. Recently, due to its simplicity, versatility and cost-effectiveness, the electrospinning technique has been widely used to fabricate nanofibers from a wide variety of materials. To our knowledge, there are very few reports available on CDs embed in one-dimensional nanofibers. Herein, we use silane pre-functionalized CDs (SiCDs) and select silane as alkoxide precursor, then the SiCD/SiO2 composite nanofibers are prepared by combining sol-gel method with electrospinning technique. The resulting product may possess new physical or chemical properties from the two components. The fluorescence quantum yield of the SiCD/SiO2 composite nanofibers is as high as 90% and the fluorescence lifetime can reach up to about 6 ns. Bioapplication experiment confirm the low cytotoxicity, well bioimaging and biocompatibility of these nanofibers. It is expected that this one-dimensional composite fibers may have more wide applications in bioimaging, textile, and optical devices for their novel geometrical structure and luminescent properties.

10077-34, Session 5

The effect of the background medium in microsphere-assisted super-resolution microscopy

Arash Darafsheh, Jarod C. Finlay, Univ. of Pennsylvania (United States)

In this work, we investigated the effect of the background medium in which the microsphere is immersed. We used finite-difference time-domain (FDTD) numerical simulation to investigate the photonic nanojet formation in the microspheres. Our results, supported by experimental observation, shows that for the same refractive index contrast high-index microsphere embedded in a low-index background medium have superior imaging capability compared to a standalone low index microsphere. Our results indicate that microsphere-assisted microscopy is a promising candidate for applications in medical and cancer research, as well as in microfluidics and nanophotonics.

10077-35, Session 5

Histological staining can enhance the performance of spectroscopic microscopy on sensing nanoarchitectural alterations of biological cells

Di Zhang, Lusik Cherkezyan, Yue Li, Ilker Capoglu, Hariharan Subramanian, Allen Taflove, Vadim Backman, Northwestern Univ. (United States)

Our group had previously established that nanoscale three-dimensional refractive index (RI) fluctuations of a linear, dielectric, label-free medium can be sensed in the far field through spectroscopic microscopy, regardless of the diffraction limit of optical microscopy. Adopting this technique, Partial Wave Spectroscopic (PWS) Microscopy was able to sense nanoarchitectural alterations in early-stage cancers. With the success of PWS on detecting cancer from healthy clinical samples, we further investigated whether and how histological staining can enhance the performance of PWS by both finite difference time domain (FDTD) simulations and experiments.

In this investigation, the dispersion models of hematoxylin and eosin were extracted from the absorption spectra of H&E stained cells. Using these models, the effect of staining were studied via FDTD simulations of unstained versus stained samples with various nanostructures. We observed that, the spectral variance was increased and the spectral variance difference between two samples with distinct nanostructures was enhanced in stained samples by over 200%. Furthermore, we investigated with FDTD whether molecule-specific staining can be used to enhance signals from a medium composing of the desired molecule. Samples with two mixed random media were created and the desired medium was either stained or unstained. Our results showed that the difference between the nanostructures of only the desired medium was enhanced in stained samples. We concluded that, with molecule-specific staining, PWS can selectively target the nanoarchitecture of a desired molecule. Lastly, these results were validated by experiments using human buccal cells from healthy or lung cancer patients.

This study has significant impact in improving the performance of PWS on quantifying nanoarchitectural alterations during cancer.

10077-36, Session 5

Laser particles as novel bioimaging agents

Nicola Martino, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Frederick Sangyeon Cho, Seok-Hyun Yun, Harvard Medical

School (United States) and Massachusetts General Hospital (United States)

Lasers are ubiquitously used in biomedical research as versatile light sources because of the wavelength selectivity, high intensity and spatiotemporal controllability they provide. However, until now, laser light has always been provided to samples from external sources. Recently, our group demonstrated that it is possible to integrate laser sources, in the form of micrometric resonators, inside living cells and biological tissues. This possibility opens the way to the development of such laser particles as optical contrast agents in bioimaging applications. In this contribution, we will explore some of the possible ways in which the peculiar properties of laser light emission from micrometric particles can be exploited to obtain new functionalities. For example, the output emission of a laser close has a strong super-linear behavior near threshold with respect to the pump power; this non-linearity allows to achieve sub-diffraction resolutions and optical sectioning capabilities. Also, given the narrow linewidth of laser emission (< 1 nm), it is possible to have a larger number of independent detection channels, compared to the broader bands of typically used fluorescent molecules and quantum dots (> tens of nm).

10077-37, Session 6

Definitive depolarization contrast for nanomedicine

Norman Lippok, Harvard Medical School (United States) and Massachusetts General Hospital (United States); Martin Villiger, Harvard Medical School (United States) and Massachusetts General Hospital (United States); Alexandre Albanese, Massachusetts Institute of Technology (United States); Eelco F. J. Meijer, Timothy P. Padera, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Sangeeta Bhatia, Massachusetts Institute of Technology (United States); Brett E. Bouma, Wellman Ctr. for Photomedicine (United States) and Massachusetts General Hospital (United States) and Harvard Medical School (United States)

The detection and visualization of nanoparticles and their distribution is a highly desirable functionality, with immediate ramification for nanomedicine. Previously, gold nanorods (NRs) have been used as exogenous contrast agents based on inherent optical properties such as differential absorption and scattering, as well as two-photon luminescence and photothermal excitation at the NR's surface plasmon resonance. NRs exhibit anisotropic properties such as dichroism that efficiently scramble the polarization state of the input light. Depolarization caused by NRs has attracted increased attention but its robust application as an image contrast remained to be demonstrated. Here, we investigate the depolarization properties of gold NRs with coherent imaging. Our studies reveal a strong dependency on the input polarization state, with circularly polarized light providing maximum depolarization. These experimental results agree with a numerical model utilizing diattenuation as the underlying mechanism for depolarization. Mixtures of NRs with non-depolarizing scatterers revealed a deterministic relation between depolarization and absolute NR concentration after correcting for the number of non-depolarizing scatterers per voxel, which is retrieved from intensity measurements. The true NR concentration could thus be retrieved with 4 pM accuracy. We also propose a Mueller matrix decomposition to extract local sample depolarization properties that are independent of the input polarization state. The depolarization contrast was validated in vivo by visualizing the collecting lymphatic vessels in a mouse foot. These results propose depolarization as a new, robust and cost efficient contrast mechanism to visualize NRs that could offer novel perspectives for studying NR transport and concentration.

10077-38, Session 6

Resolution enhancement for deep tissue imaging with plasmonic saturated excitation microscopy

Gitanjal Deka, Hou-Xian Ding, Kuan-Yu Li, Shi-Wei Chu, National Taiwan Univ. (Taiwan)

A major challenge in tissue imaging is the degradation of resolution with increased depth, due to multiple scattering and refraction. By using long-wavelength lasers, penetration depth can be improved, while resolution further degrades. Recently, superresolution techniques emerged to enhance spatial resolution by switching or saturation of fluorescence. However, fluorescence suffers from photobleaching, and current techniques do not provide deep-tissue imaging capability due to the lack of optical sectioning or the requirement of special beam manipulation.

We have recently demonstrated that scattering from a single gold nanoparticle exhibits saturation behavior, which was adopted to significantly enhance resolution by saturated excitation (SAX) microscopy. Compared to fluorophores, scattering from plasmonic nanoparticles is free from bleaching, the cross-section is much larger, and the plasmonic resonance band is broadly tunable with particle shape and size, making it an ideal and robust contrast agent for long-term observation. On the other hand, SAX microscopy does not need any beam engineering and provides intrinsic sectioning with its confocal scheme, suitable for deep-tissue imaging.

In this work, we combine the advantages of plasmonics and SAX microscopy to demonstrate resolution enhancement underneath a very deep tissue. One general concern of scattering-based imaging is the background from the strong scattering of the surrounding tissue. Since tissue scattering is linear, and SAX allows the extraction of only nonlinear responses, the background can be fully eliminated, leaving only nanoparticle visible. Therefore, such combination provides a novel tool for not only high-resolution, but also high-contrast, background-free, and long-term imaging deep inside biological tissues.

10077-39, Session 6

A smartphone compatible colorimetric biosensing system based on porous silicon

Tengfei Cao, Yiliang Zhao, Sharon M. Weiss, Vanderbilt Univ. (United States)

Smartphones have the potential to become an excellent platform for point-of-care diagnostic devices with their multiple sensors that can serve as a test kit. In this paper, we report a smartphone-compatible, cost-effective, colorimetric biosensing system based on a porous silicon (PSi) rugate filter. The sensor was initially characterized by using an optical microscope equipped with a digital camera that acquired images of the PSi sample upon attachment of different concentrations of glucose molecules. When molecules infiltrate into the pores, the effective refractive index of PSi increases, causing the peak reflectance wavelength of the rugate filter to redshift. To facilitate color-based detection, a narrow bandwidth, 10 nm FWHM bandpass filter with peak transmittance at 610 nm was utilized to correlate the wavelength shift with intensity changes in the RGB color of the images. An algorithm was developed to remove the noise in the images, which improved both the sensitivity and reliability of the colorimetric detection. Finally, a linear relationship was found between the change of red intensity in the RGB model and the wavelength shift. The minimum resolvable wavelength shift turned out to be less than 0.25 nm, which is comparable with the detection limit of many commercial spectrophotometers. The porous silicon rugate filter demonstrates a sensitivity of 310 nm/RIU for solution-based measurements, which corresponds to a detection limit near 7?10^-4 RIU. By utilizing a smartphone camera LED and smartphone camera as the light source and detector, respectively, this system holds promise as a low-cost point-of-care diagnostic tool.

10077-40, Session 6

Effects of cholesterol depletion on membrane nanostructure in MCF-7 cells by atomic force microscopy

Yuhua Wang, Fujian Normal Univ. (China)

The cell membrane is composed of phospholipids, glycolipid, cholesterol and proteins that are dynamic and heterogeneous distributed in the bilayer structure and many researches have showed that the plasma membrane in eukaryotic cells contains microdomains termed “lipid raft” in which cholesterol, sphingolipid and specific membrane proteins are enriched. Cholesterol extraction induced lipid raft disruption is one of the most widely used methods for lipid raft research and M?CD is a type of solvent to extract the cholesterol from the cell membrane. In this study, the effect of M?CD treatment on the membrane nanostructure in MCF-7 living cells was investigated by atomic force microscopy. Different concentrations of M?CD were selected to deplete cholesterol for 30 min and the viability of the cells was tested by MTT assay to obtain the optimal concentration. Then the morphology and nanostructure of the cell membrane were detected. The results show that appropriate concentration of M?CD can induce the alteration of the cell morphology and the roughness of membrane surface decrease significantly. This may indicate that the microdomains of the cell membrane disappear and the cell membrane appears more smoothly. Cholesterol can affect nanostructure and inhomogeneity of the plasma membrane in living cells.

10077-41, Session 6

Nanoscopy of cell cytoskeleton in hypoxic HeLa cells

Xiao Peng, Shuyi Yuan, Danying Lin, Wei Yan, Zhigang Yang, Rui Hu, Jing Qi, Junle Qu, Shenzhen Univ. (China)

Recently, super-resolution fluorescence microscopy, including Stochastic optical reconstruction microscopy (STORM), has been used to obtain images of cells at nanometer scale, providing more detailed information of cellular structural features smaller than 200 nm that were difficult to observe by traditional light microcopy. Among these cellular structures, cell cytoskeleton plays an essential role in multiple important cell physiological processes, such as cell migration, invasion and metastasis. The mutations of cytoskeleton are considered to be associated with the chemotherapy resistance of cancer cells. However, the hypoxia induced changes of cell actin cytoskeleton and its interacting partners are still not clear. In this study, we developed a STORM system and applied this system to observe the cytoskeleton of HeLa cells under either normal or hypoxic conditions. As a result, morphological changes of the actin cytoskeleton have been observed in hypoxic HeLa cells, indicating that changes in cytoskeleton structure might contribute to the phenotypic and functional changes in solid human tumor cells.

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10078-1, Session 1

Recent insights in the spectroscopic properties of upconversion nanoparticles *(Invited Paper)*

Marco Kraft, Christian Würth, Martin Kaiser, Bundesanstalt für Materialforschung und -prüfung (Germany); Verena Muhr, Thomas Hirsch, Univ. Regensburg (Germany); Ute Resch-Genger, Bundesanstalt für Materialforschung und -prüfung (Germany)

Lanthanide-doped up-converting nanoparticles (UCNPs) are promising reporters for medical diagnostics and bioimaging, which are excited in the near infrared (NIR) by multiphoton absorption processes, and show multiple narrow emission bands in the visible (vis) and NIR, long luminescence lifetimes in the μs range, and excellent photostability, [1,2]. Current limitations present their relative low absorption cross sections and low fluorescence efficiencies, with the latter being affected by particle size, surface chemistry, and microenvironment, particularly water [3-5].

Here, we present results from systematic studies of the excitation power density dependent upconversion luminescence spectra, intensities/intensity ratios of the individual emission bands, slope factors, and quantum yields of UCNPs of varying size, dopant concentration, and surface chemistry in different microenvironments as well as the up- and downconversion luminescence decay kinetics of the different emission bands [6]. Moreover, first studies of the energy transfer from UCNPs to surface-bound organic dyes acting as fluorescence acceptors are shown [6]. Based upon these measurements, fluorescence deactivation channels are identified and spectroscopic parameters for the screening of material performance are derived.

10078-3, Session 1

All-semiconductor near-infrared to visible upconversion nanoparticles

Dan Oron, Ayelet Teitelboim, Weizmann Institute of Science (Israel)

Upconversion (UC) is a nonlinear process in which two, or more, long wavelength photons are converted to a shorter wavelength photon. This process is based on sequential absorption of two or more photons, involving metastable, long lived intermediate energy states, thus is not restricted to ultrashort pulsed excitation. Hence, requirements for UC processes are long lived excited states, a ladder like arrangement of energy levels and a mechanism inhibiting cooling of the hot charge carrier. UC holds great promise for bioimaging, enabling to perform multiphoton imaging in scattering specimen at very low powers. Rare-earth-doped nanocrystals, the most commonly used ones for UC, typically require a minimal particle diameter of several tens of nanometers and have a limited action spectrum. Here, we present a novel luminescence upconversion nano-system based on colloidal semiconductor double quantum dots, consisting of a NIR-absorbing component and a visible emitting component separated by a tunneling barrier in a spherical onion-like geometry. These dual near-infrared and visible core/shell/shell PbSe/CdSe/CdS nanocrystals are shown to efficiently upconvert a broad range of NIR wavelengths up to 1.2 microns to visible emission at room temperature, covering a spectral range where there are practically no alternative upconversion systems. The particle diameter is less than ten nanometers, and the synthesis enables versatility and tunability of both the visible emission color and the NIR absorption edge. The physical mechanism for upconversion in this type of structures, as well as potential advances and extensions on this system will be discussed.

10078-4, Session 1

Lanthanum fluoride upconverting nanoparticles for photo-biomodulation of cell function

Sumeyra Tek, Ashley N. Tran, L. Christopher Mimun, Brandy K. Vincent, Kelly L. Nash, The Univ. of Texas at San Antonio (United States)

Inorganic fluorescent nanoprobes have been widely used as passive agents for intracellular imaging for decades. An emerging field of research is the development of these contrast agents and using them actively in a way that they respond external stimulation by inducing photo-chemical, thermal or mechanical actions that enable control and modulation over cell function. To achieve such control, methods which are remote, non-invasive and with low-thermal means of stimulation is preferable. Among a large variety of candidates, lanthanide doped upconverting nanoparticles (UCNPs) are one of the most interesting class of fluorescent materials. Non-scattering, low energy near infrared (NIR) light can be used for excitation of UCNPs as on-demand light sources resulting in emission peaks throughout the near-UV and visible wavelengths. Towards this goal we developed nano-size, hydrophilic, non-toxic and biocompatible core/shell nanoparticles with enhanced upconversion intensity for photobiomodulation studies. Under this approach, undoped LaF3 (inert) shell and Yb3+ doped LaF3 (active) shell are grown on core LaF3:20%Yb, 2%Tm upconverting nanoparticles for enhanced luminescence first time with rapid and green microwave-assisted synthesis method that employs Polyvinylpyrrolidone (PVP) as biocompatible surfactant. The as-synthesized high efficiency UCNPs (10-25 nm for core and core/shell) are analyzed through XRD, TEM, HRTEM, and Photoluminescence spectrum is acquired under 980 nm laser excitation. Cytotoxicity measurements show high levels of viability upon nanoparticle uptake by ARPE-19 cells. Confocal microscopy is used to visualize nanoparticles in cells. The results of this study form the basis of understanding the combined effects of upconverted light and laser excitation on cell function.

10078-5, Session 2

Development and characterization of magnetic-fluorescent nanostructures as combined nanoheaters and nanothermometers

Corneliu I. Rablau, Zhenyuan Zhang, Ronald E. Kumon, Ronald J. Tackett, Uma Ramabadran, Prem P. Vaishnava, Kettering Univ. (United States)

We report on the development and characterization of mesoporous silica nanostructures (MSN, ~ 50 to 100 nm) embedded with both superparamagnetic iron oxide nanoparticles (SPIONS, ~ 5 to 10 nm) and with Er3+,Yb3+-NaYF4 nanocrystals (~ 5 to 10 nm). Such structures are intended as combined nanoheaters and nanothermometers in applications requiring simultaneous heat generation and temperature measurement at the cellular and sub-cellular level, such as in studies of temperature-dependent intracellular processes, heat-activated targeted drug delivery or targeted magnetic-hyperthermia treatment of tumors. The heat is generated by the embedded ?-Fe2O3 and Fe3O4 SPIONS under an externally-applied RF magnetic field (~ 250 Gauss, ~ 150 kHz) via Neel relaxation. The Er3+- and Yb3+ - doped NaYF4 nanocrystals provide the temperature-sensitive fluorescent component. Indeed, the upconversion fluorescence spectrum of these nanocrystals has two green emission bands (around 525 nm and 540 nm respectively). The intensity ratio of these two fluorescence bands is a function of temperature, but is independent of the total fluorescence intensity. This ratiometric principle of temperature measurement has the

advantage of absolute measurement and high sensitivity, and is less subject to environmental perturbations. MSNs were chosen as the embedding medium because they are studied extensively as a biocompatible platform for hyperthermia and drug delivery, thus providing a perfect match for our intended applications. We investigate the SPIONS, the Er3+,Yb3+-NaYF4 nanocrystals and the resulting mesoporous silica nanostructures via Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD), temperature-dependent fluorescence (photoluminescence) and RF-magnetic-field-field induced hyperthermia.

10078-6, Session 2

Effects of iron-oxide nanoparticles on oral biofilms

Jane Q. Nguyen, Gema J. Alas, Ronald E. Pagano, Diana B. Aboytes, Christina Calleros, Martha D. Sherlin, The Univ. of New Mexico (United States); H. M. H. Nihal Bandara, The Univ. of Queensland (Australia); Sergei A. Ivanov, Los Alamos National Lab. (United States); Gennady A. Smolyakov, Christine N. Nathe, The Univ. of New Mexico (United States); Dale L. Huber, Sandia National Labs. (United States); Hugh D. C. Smyth, The Univ. of Texas at Austin (United States); Marek Osi?ski, The Univ. of New Mexico (United States)

Human mouth is a host of a large gamut of bacteria species, with over 700 of different bacteria strains identified. Most of these bacterial species are harmless, some are beneficial (such as probiotics assisting in food digestion), but some are responsible for various diseases, primarily tooth decay and gum diseases such as gingivitis and periodontitis. For example, Streptococus mutans produces enamel-eroding acids, while Porphyromonas gingivalis is strongly linked to periodontitis. In this paper, we report on the effects of exposure of oral biofilms to iron oxide nanoparticles as possible bactericidal agent.

10078-7, Session 2

A green approach for the fabrication of near infrared absorbing iron oxide nanoparticles for biomedical applications

Prashant Kharey, Anshu Kumari Mishra, Gaurishankar Shaw, Saumya Jaiswal, Sharad Gupta, Indian Institute of Technology Indore (India)

Nanomaterials exhibit fascinating physical, chemical, electronic and magnetic properties. Some nanomaterials occur naturally, but of particular interest are engineered nanomaterials, which are designed and being used in many commercial products and processes. Among the various nanomaterials, magnetic nanoparticles are of great interest due to their potential applications in the field of healthcare, especially in targeted magnetic resonance imaging due to deep tissue imaging ability. The chemical and physical properties of the engineered nanoparticles can be tailored by controlling their size and surfaces. The aim of this study was to synthesize biocompatible iron oxide nanoparticles with a controllable size distribution and band gap using green chemistry based approach. This is a simple facile and efficient single step approach to synthesize multifunctional (magnetic, photo acoustic and photo thermal agent) iron oxide nanoparticles. In this fabrication of these nanoparticles no synthetic reducing agents were used for the reaction. A natural precursor, extracted from medicinal plant found in India such as Neem (Azadiracta Indica), was used in the fabrication of these nanoparticles. In addition, these particles are endowed with the ability of surface functionalization. The optical properties of these green chemistry based iron oxide nanoparticles are tuned into tissue transparent near infrared (NIR) region by changing their morphology. Eventually proposed iron oxide nanoparticles may be used for multi-modal deep tissue imaging and therapeutics.

10078-9, Session 3

Optical steering of metallic nanoparticles using 3D microbubbles

Ujitha A. Abeywickrema, Arjun Krishnappa, Partha P. Banerjee, Univ. of Dayton (United States); Uttam Sinha, Keck Medicine of USC, The Univ. of Southern California (United States)

In our previous work, induced force on a microbubble due to a focused laser beam is studied by deriving a general force model. It is also shown that the microbubble can be steered using a low power laser beam. Nanoparticles are agglomerated around microbubbles when they are mixed together and this is investigated using silver nanopartilcles in our previous work. These nanoparticle-surrounded microbubbles can be used to deliver nanoparticles to a desired location which has potential application in drug delivery systems. When a microbubble is surrounded by nonoparticles, the total induced force due to a focused laser beam can be considered as the sum of the force on the microbubble and the force on nanoparticles. Nanoparticles can be considered as induced dipoles in the presence of an optical field. Thus the total force due to nanoparticles can be calculated by adding the forces induced on each nanoparticle. An equivalent force for a microbubble surrounded by nanoparticles is also calculated and presented in our previous work. All these models are developed by assuming the bubble to have a 2d geometry and assuming a single transverse dimension for the propagating laser beam. In this work, the total force induced on a nanoparticle-surrounded microbubble is calculated using a three-dimensional geometry and a circularly symmetric Gaussian beam. Different types and sizes of nanoparticels are also investigated. Effect of viscosity on microbubble dynamics is also discussed.

10078-10, Session 3

Photochemical generation of antimicrobial Ag-nanoparticles in intraocular lenses

Thorben Badur, Hee-Cheol Kim, Norbert A. Hampp, Philipps-Univ. Marburg (Germany)

The antimicrobial properties of silver (Ag) nanoparticles (NP) have been investigated in depth during the last decades.[1] For cataract treatment minimal invasive surgery has become state-of-the-art. The physicians are still fighting against postoperative inflammations, such as endophthalmitis.[2] We present a novel approach to reduce these postoperative complications by equipping the hydrophilic intraocular lenses (IOL) with a Ag NP depot. As the Ag NP are completely entrapped inside the polymeric IOL no direct contact of the nanoparticles with epithelial cells may occur.

Using 1-hydroxybenzotriazole (HOBt) or 7-hydroxycumarine (HOCum) as photo reduction mediators (PRM) the formation of the Ag NP is accomplished in situ. PRM and Ag nitrate are diffused into the ready made IOL. By means of two-photon-absorption (TPA) photochemistry at ?TPA = 532 nm the AG NP generation is precisely controlled to occur inside the IOL only. At no point NP are directly exposed to the surface.[3] Interesting dependencies between the used PRM and the resulting particle size distribution or the effectiveness of the silver ion reduction inside the polymer matrix are reported. The Ag NP were prepared in the outer area of the IOL not to affect the optical properties of the ophthalmic implant. The amount of Ag ions released was determined and found to be sufficient to effectively reduce the counts of airborne germs.

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10078-11, Session 3

Nanoemulsion structural design for cancer delivery of hybrid fluorophores

Urszula Bazylinska, Dominika Wawrzynczyk, Wroclaw Univ. of Science and Technology (Poland); Julita Kulbacka, Wroclaw Medical Univ. (Poland)

Effective nanocarriers (NCs) for cancer treatment need both, high colloidal stability and biocompatibility, to achieve high drug delivery to the cancer cells, avoiding rapid clearance by the reticuloendothelial system and cytotoxicity to normal cells. Recently the field of drug encapsulation has raised much interest due to the advancement of the biomaterials used to elaborate the capsules with novel functional properties obtained by nanoemulsion structural design. Among those approaches, an application of a hybrid cargo as structural matrix for co-precipitating with polymer in the micellar pseudophase known to the embedding process, is considered to be one of the most promising because it permits the encapsulation both hydrophilic and hydrophobic compounds.

In the present study poly(lactide-co-glycolide and poly(caprolactone) biocompatible polyesters also grafted with poly(ethylene glycol)-for fabrication of stealth NCs, have been used in nanoemulsion embedding process for co-encapsulation luminescent lanthanide-doped nanocrystals - NaYF4:Tm3+,Yb3+ and organic porphyrin-origin photosensitizing dye, to applied them as multifunctional hybrid agents for theranostic purposes in human malignant melanoma (MEWO and Me45) cells. After the optimization process by DLS, ?-potential, TEM, AFM, backscattering, fluorescent spectroscopy, spherical polyester NCs with average size < 200 nm were chosen for evaluation of the therapeutic effect based on, cytotoxicity, photoactivity and bioimaging studies on the skin cancer cells, normal keratinocytes (HaCaT) and also macrophage cell line to test the effectiveness of the stealth NCs. Our investigations proved that, by rational designed nanoemulsion structural technique it is possible to obtain biocompatible nanocarriers with enhanced internalization and photoactivity of hybrid fluorophores in skin cancer cells.

10078-13, Session 4

Mucin1 antibody-conjugated dye-doped mesoporous silica nanoparticles for breast cancer detection in vivo

Juan L. Vivero-Escoto, The Univ. of North Carolina at Charlotte (United States) and The Ctr. for Biomedical Engineering and Science, The Univ. of North Carolina at Charlotte (United States); Laura Jeffords Moore, Didier Dreau, Mubin Tarannum, Merlis P. Alvarez-Berrios, Pinku Mukherjee, The Univ. of North Carolina at Charlotte (United States)

The development of novel methods for tumor detection is a burgeoning area of research. In particular, the use of silica nanoparticles for optical imaging in the near infrared (NIR) represents a valuable tool because their chemical inertness, biocompatibility, and transparency in the ultraviolet-visible and NIR regions of the electromagnetic spectrum. Moreover, silica nanoparticles can be modified with a wide variety of functional groups such as aptamers, small molecules, antibodies and polymers. Here, we report the development of a mucin 1(MUC1)-specific dye-doped NIR emitting mesoporous silica nanoparticles (MUC1-NIR-MSN) platform for the optical detection of breast cancer tissue overexpressing human tumor-associated MUC1. We have characterized the structural properties and the in vitro performance of this system. The MSN-based optical imaging probe is non-cytotoxic and targets efficiently murine mammary epithelial cancer cells overexpressing human MUC1. Finally, the ability of MUC1-NIR-MSN contrast imaging agent to selectively detect breast cancer tumors overexpressing human tumor-associated MUC1 was successfully demonstrated in a transgenic murine mouse model. The NIR imaging experiments on tumor-bearing animals showed specific accumulation of the MSN-based probe in human MUC1-positive tumors and small signal in control tumors. We envision that this MUC1-specific MSN-based optical probe has the potential to greatly aid in screening prospective patients for early breast cancer detection and in monitoring the efficacy of drug therapy.

10078-14, Session 4

Novel laser-synthesized Si-based nanomaterials for cancer theranostics

Andrei V. Kabashin, Lasers, Plasmas et Procédés Photoniques (France)

The presentation will overview our on-going activities on laser ablative synthesis of Si-based nanomaterials and their testing in biomedical tasks. Our approach is based on ultra-short (fs) laser ablation from a solid target or already formed water-suspended colloids to achieve an efficient control of size characteristics of “bare” ligand-free nanomaterials, or fabricate nanomaterials coated by functional biopolymers (dextran, PEG) to minimize immune response of biological systems. Our experiments in vitro demonstrate an excellent cell uptake of both bare and functional nanomaterials, while the composition of protein corona covering nanoparticles complexes in biological environment promises a good transport of nanomaterials in vivo. In addition, the intravenous administration of Si NPs using small animal model did not reveal any toxicity effects, which was confirmed by behavior of mice, stability of blood content and other biochemical parameters, as well as by histology analyses of all organs and biodistribution of nanoparticles in tissues. Furthermore, the nanoparticles rapidly biodegraded in the organism and were completely cleared 2-3 days after their injection. In general, our tests evidenced a negligible toxicity and much faster clearance of laser-synthesized Si NPs compared to all chemically-synthesized Si NPs counterparts. Laser-synthesized nanomaterials are now actively tested in cancer diagnostics and therapy (theranostics) tasks. In particular, our experiments showed that laser-synthesized nanomaterials can provide a much better efficiency compared to chemically synthesized counterparts in a newly introduced method of mild cancer therapy using Si nanoparticles as sensitizers of radiofrequency radiation-based hyperthermia, as well as be efficient markers for bioimaging.

10078-15, Session 4

Effects of cancer cell permeability control on the efficiency of cell damage through surface plasmon resonance of gold nanoparticle

Jen-Hung Hsiao, Jian-He Yu, National Taiwan Univ. (Taiwan); Yulu He, Xi’an Jiaotong Univ. (China) and National Taiwan Univ. (Taiwan); Yi-Chou Tu, Wei-Hsiang Hua, Meng Chun Low, Cheng-Che Hsieh, Yean-Woei Kiang, Chih-Chung Yang, National Taiwan Univ. (Taiwan)

Cancer cell killing efficiencies based on the photothermal effect caused by the surface plasmon resonance of metal nanoparticles (NPs) and the photodynamic effect caused by the singlet oxygen generation of a photosensitizer rely on the cell uptake efficiency of metal NP and photosensitizer. Perforation and heating can increase cell membrane permeability and hence can increase the cell uptake efficiency of NPs and drugs. In this paper, we demonstrate the variations of the cell damage efficiency under the illuminations of different lasers, which can produce mainly photothermal effect, mainly photodynamic effect, and mixed

effect, when a pre-perforation and a pre-heating processes are applied. Au nanorings (NRIs) with their localized surface plasmon resonance wavelength around 1064 nm are used. The perforation process is undertaken by illuminating the cell samples by a femtosecond laser at 1064 nm with the power density lower than the cell damage threshold intensity. The heating process is implemented by illuminating cells with a low power continuous laser at 1064 nm. It is found that with the pre-perforation and pre-heating processes, the photodynamic effect is enhanced because the internalized Au NRI number and hence the internalized photosensitizer (AlPcS) molecule number are increased. However, the photothermal effect can be reduced because the adsorbed Au NRIs on cell membrane are effectively internalized during the pre-perforation and pre-heating processes. The photothermal effect is more effective when Au NRIs are adsorbed on cell membrane.

10078-16, Session 4

Ultrasound-guided photoacoustic imaging of lymph nodes with biocompatible gold nanoparticles as a novel contrast agent

In-Cheol Sun, Diego Dumani, Stanislav Y. Emelianov, Georgia Institute of Technology (United States)

A key step in staging cancer is the diagnosis of metastasis that spreads through lymphatic system. For this reason, researchers develop various methods of sentinel lymph node mapping that often use a radioactive tracer. This study introduces a safe, cost-effective, high-resolution, high-sensitivity, and real-time method of visualizing the sentinel lymph node: ultrasound-guided photoacoustic (US/PA) imaging augmented by a contrast agent. In this work, we use clearable gold nanoparticles covered by a biocompatible polymer (glycol chitosan) to enhance cellular uptake by macrophages abundant in lymph nodes. We incubate macrophages with glycol-chitosan-coated gold nanoparticles (0.05 mg Au/ml), and then fix them with paraformaldehyde solution for an analysis of in vitro dark-field microscopy and cell phantom. The analysis shows enhanced cellular uptake of nanoparticles by macrophages and strong photoacoustic signal from labeled cells in tissue-mimicking cell phantoms consisting gelatin solution (6 %) with silica gel (25 ?m, 0.3%) and fixed macrophages (13 X 105 cells). The in-vivo US/PA imaging of cervical lymph nodes in healthy mice (nu/nu, female, 5 weeks) indicates a strong photoacoustic signal from a lymph node 10 minutes post-injection (2.5 mg Au/ml, 80 ?l). The signal intensity and the nanoparticle-labeled volume of tissue within the lymph node continues to increase until 4 h post-injection. Histological analysis further confirms the accumulation of gold nanoparticles within the lymph nodes. This work suggests the feasibility of molecular/cellular US/PA imaging with biocompatible gold nanoparticles as a photoacoustic contrast agent in the diagnosis of lymph-node-related diseases.

10078-17, Session 4

Photothermal technique to damage cancer cells: in vitro studies of simultaneous pulsed and cw irradiation

Noe Zamora-Romero, Guillermo Aguilar, Luis F. Devia-Cruz, Univ. of California, Riverside (United States); Darren Banks, California State Univ., Fullerton (United States); Bin Zhang, David L. Halaney, Univ. of California, Riverside (United States)

Laser-nanoparticles interactions have been widely used for several years. In biomedicine, several in vitro and in vivo experiments have shown promising results for the detection and treatment of cancer. One of the techniques of interest to us, is the nanoparticle-assisted photothermal therapy (PTT), which consists of irradiating cancer cells incubated with nanoparticles with either a pulsed or continuous (cw) laser in order to damage the cells.

However, there is still a debate over which type of laser is most effective for PTT for cancer treatment. On the one hand, cw lasers are minimally invasive and can be used for both detection and treatment of tumors. On the other hand, pulsed lasers offer great spatial precision and can deposit higher energy fluences than cw lasers, making them very efficient for inducing cavitation to damage cancer cells and tumors mechanically.

The aim of this study is to investigate whether simultaneous application of cw and pulsed laser could offer a synergetic enhancement of PTT efficacy to damage cancer cells in vitro, compared to either laser applied individually. PTT efficacy is evaluated through cell viability tests following the irradiation of prostate cancer (PC3) cells incubated with gold nanorods (5.7 X10 10 p/ml).

By irradiating the PC3-nanorod solution with the cw laser at 808 nm for ~60 seconds, the temperature increases from 37.5 to ~45°C, which damages some cancer cells via the heat shock response within the cells, and also could increase their sensitivity to the mechanical stress caused by the shock wave generated from inducing cavitation in the solution by the pulsed laser irradiation.

10078-18, Session 5

Plasmonic copper chalcogenides as multifunctional theranostics platform for photoacoustic imaging and photothermal therapy *(Invited Paper)*

Junjie Zhu, Nanjing Univ. (China)

Localized surface plasmon resonances arising from the free carriers in copper-deficient copper chalcogenides nanocrystals (Cu2-xE, E=S,Se) enables them with high extinction coefficient in the near-infrared range, which was superior for photothermal related purpose. Although Cu2-xE nanocrystals with different compositions (0< x?1) all possess NIR absorption, their extinction coefficients were significantly different due to their distinct valence band free carrier concentration. Herein, by optimizing the synthetic conditions, we were able to obtain pure covellite phase CuS nanoparticles with maximized free carrier concentration (x=1), which provides extremely high mass extinction coefficient (up to 60 Lg-1cm-1 at 980 nm and 32.4 Lg-1cm-1 at 800 nm). To the best of our knowledge, these values was maximal among all inorganic nanomaterials. High quality Cu2-xSe can also be obtained with a similar approach. In order to introduce CuS nanocrystals for biomedical applications, we further transferred these nanocrystals into aqueous solution with an amphiphilic polymer and colvalently linked with beta-cyclodextrin. Using host-guest interaction, adamantine-modified RGD peptide can be further anchored on the nanoparticles for the recognition of integrin-positive cancer cells. Together with the high extinction coefficient and outstand photothermal conversion efficiency (determined to be higher than 40%), these CuS nanocrystals were applied for photothermal therapy of cancer cells and photoacoustic imaging. In addition, anticancer drug doxorubicin can also be loading onto the nanoparticles through either hydrophobic or electrostatic interaction for chemotherapy.

10078-19, Session 5

Plasmonic-based nanoprobes for dynamic sensing of single tumor cells

Zixuan Chen, Nanjing Univ. (China)

We described here two plasmonic-based nanoprobes with purpose of imaging dynamic biologic process of single tumor cells. At first, we proposed a multi-modified core-shell gold@silver nanorods for real-time monitoring the entire autophagy process at single-cell level. Autophagy is vital for understanding the mechanisms of human pathologies, developing novel drugs and exploring approaches for autophagy controlling. The plasmon resonance scattering spectra of the nanoprobes was superoxide radicals (O2•-)-dependent, a major indicator of cell autophagy, and suitable for real-time monitoring at single-cell level. More importantly, with the introduction of 'relay probe' operation, two types of O2•--regulating

autophagy processes were successfully traced from the beginning to the end, and the possible mechanism was also proposed. According to our results, intracellular O2•- level controlled the autophagy process by mediating the autolysosome generation. Different starvation approaches can induce different autophagy processes, such as diverse steady state time-consuming. In addition, a plasmonic-based nanothermometer was prepared via dense thermosensitive polymer (pNIPAAm) capping on gold nanorods, of which the plasmon resonance spectra was linearly dependent on adjacent temperature. In this work, the white light transmitted dark-field illuminator was replaced by a laser total internal reflection dark-field microscope (LTIR-DFM) system in order to overcome the low-throughput and inexorable biological scattering background of DFM, as well as interference from mechanic noise, nanoprobe direction, optical system drift, etc. With this nanothermometer, we have successfully captured temporal biological thermal process (thermogenesis) occurred in single tumor cells, providing a new potential strategy for in-situ cellular analysis.

10078-21, Session 6

TiO2 nanoparticles for enhancing the refractive index of hydrogels for ophthalmological applications *(Invited Paper)*

Norbert A. Hampp, Thorben Badur, Christian Dams, Hendrik M. Reinhardt, Philipps-Univ. Marburg (Germany)

Intraocular lenses (IOL) are currently the only treatment cataract dependent vision impairment and blindness.[1] A polymer suitable for IOL manufacture needs to meet a plurality of properties, biocompatibility, excellent transmission in the visible range, a high flexibility for micro invasive surgery, a high refractive index as well as a good ABBE-number, just to mention the most important ones.[2]

We present the use of in situ generated TiO2-nanoparticles to enhance the refractive index of poly-HEMA hydrogels - which are suitable polymers for IOL manufacture[3] – from 1.44 to 1.527 at 589.3 nm combined with an excellent a ABBE-number of 54. The nanoparticles were prepared using titanium diisopropoxide bis(acetylacetonate) as a precursor. First the titanium salt was diffused into the poly-HEMA matrix and then it was transformed into TiO2 in boiling water. The resulting TiO2-poly-HEMA hydrogel was dried for 10 days under ambient conditions. By lathing these polymers were machined into lens precursors, the so-called Saturn-rings. After re-swelling in physiological saline solutions flexible polymer lenses with high surface quality, shape memory and superior optical properties were obtained. The crystal structure of the formed TiO2 nanoparticles was identified as anatase via X-ray. No release of titanium ions or TiO2 nanoparticles was observe under physiological conditions. Such hybrid materials of TiO2 nanoparticles and poly-HEMA like hydrogels are promising materials for IOL.

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10078-22, Session 6

Dual activity of carbon nanotube/CpG: inhibition of glioma migration and stimulation of macrophages

Jacob M. Berlin, City of Hope Beckman Research Institute (United States)

Even when treated with aggressive current therapies, most patients with glioblastoma survive less than two years. We previously reported that single-walled carbon nanotubes (SWCNTs) can be used to dramatically increase the immunotherapeutic efficacy of CpG oligonucleotides in a mouse model of glioma. Recently we showed that the activity observed was due to a complex mixture. Here we show that a simplified SWCNT/CpG construct not only maintains its immunostimulatory property and enhances the migration of macrophages, but surprisingly also inhibits the migration of glioma cells.

10078-23, Session 6

Quantum dot-based bioconjugates for real-time imaging of cellular membrane potential

James B. Delehanty, Michael H. Stewart, Okhil Kumar Nag, Jeffrey Deschamps, Kimihiro Susumu, Eunkeu Oh, Lauren D. Field, Alexander L. Efros, Alan L. Huston, Igor L. Medintz, U.S. Naval Research Lab. (United States)

With the advent of the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative announced in 2013, the goal is to map the interconnections of the brain's tens of billions of neurons. As a result, significant interest has arisen in the development of new optical probes for the visualization of the membrane potential/action potential activity of large numbers (hundreds to thousands) of neurons simultaneously with single cell resolution. Ideally, such probes would overcome some of the limitations of current materials and techniques. Patch clamp, for example, is limited to single cell analysis while voltage-sensitive dyes and fluorescent proteins are plagued by photobleaching and intracellular instability. Semiconductor nanocrystals or quantum dots (QDs) have emerged as superior optical probes for cellular applications given their photostability, amenability to bioconjugation, and their ability to engage in efficient energy and electron transfer. We have developed a voltage-sensitive QD-based assembly comprised of a central QD scaffold around which is appended multiple copies of a peptide-fullerene conjugate. The photoexcited QD engages in electron transfer to the fullerene electron acceptor and the rate of this transfer process is modulated by changes in cellular membrane potential. We show the ability to control the efficiency of electron transfer by ratiometrically controlling the number of fullerene electron acceptors arrayed around the QD as well as by controlling the distance of the fullerene from the QD surface. In HeLa cells and in primary mouse cortical neurons, this optical construct efficiently reports on changes in cellular membrane potential induced by potassium chloride depolarization. The implications of this sensing modality on the ability to visualize the activity of neuronal cells, and other electrically excitable cells, will be discussed.

10078-24, Session 7

Using giant nanocrystal quantum dots (g-NQDs) for the Förster resonance energy transfer (FRET)-based sensing of small molecule analytes *(Invited Paper)*

Margaret Chern, Thuy Nguyen, Allison M. Dennis, Boston Univ. (United States)

It is qualitatively known that core/shell heterostructured quantum dots (QDs) with thick-shells (so-called 'giant' nanocrystal quantum dots, or g-NQDs) are more chemically robust and brighter than traditional, thin-shelled QDs. In this work, we quantify the value of extra shell layers in biosensing and bioimaging applications in traditional and cadmium-free g-NQDs. The molar cross-sections and quantum yields of a variety of g-NQDs in hexane and water are determined in order to quantify the brightness of QDs of various shell thicknesses. In addition, their effectiveness as energy donors in Förster Resonance Energy Transfer

(FRET)-based biosensors is systematically assessed. We find that thick shelled QDs exhibit extreme brightnesses, largely owing to enormous molar extinction coefficients. While FRET efficiencies decrease as expected with thicker shells due to an increase in the donor-acceptor distance, energy transfer is still evident even using g-NQDs with shells eighteen atomic monolayers thick. We systematically investigate the appropriateness of the point-dipole approximation for the calculation of donor-acceptor distance for Quasi-Type-II QDs in light of the spreading of the exciton into the shell material and find the assumption to be accurate across our range of shell thicknesses. The exceptional brightnesses of the g-NQDs yield extremely sensitive biosensors for small molecule analytes when paired with protein and DNA binding pairs. Our unique design for the label-free detection of small-molecule analytes benefits from the combination of bright g-NQD donors and novel biological parts mined from microbiology.

10078-25, Session 7

Quantum rods as efficient energy acceptors in bioluminescence resonance energy transfer *(Invited Paper)*

Mathew Maye, Syracuse Univ. (United States)

Research at the nanoscale biotic-abiotic interface centers on endowing a nano-object with the physical, chemical, or energetic properties of bio systems. We recently showed that bioluminescence resonance energy transfer (BRET) between Photinus pyralis (Ppy) firefly luciferase and core/shell semiconductive quantum rods (QRs) is particularly efficient (Nano Letters 2012, 12, 3251; Nanoscale 2013, 5, 5303; ACS Nano 2016, 10, 1969) compared to smaller quantum dots (QDs). In this presentation we will describe recent experiments designed to elucidate the primary reason for this. We studied BRET as a function of QR absorption properties, as well as rod aspect ratio and internal microstructure, and results indicate that a specific rod type is optimized for acting as energy acceptors during transfer. Interestingly, BRET efficiency was also found to decreases with increased Ppy:QR loading, a finding that suggests that the first few proteins bind to specific areas of the rods, which we believe to be defect rich regions of the interface. The results were analyzed via Forster theory, lifetime measurements, fluorescence anisotropy measurements, and single-particle spectroscopy. These results, potential photonic applications, and future design criteria needed to increase both efficiency and brightness will be discussed. We thank the AFOSR for support of this work (FA9550-10-1-0033).

10078-26, Session 7

Semiconductor quantum dots as Förster resonance energy transfer donors for intracellularly-based biosensors

Lauren D. Field, Scott A. Walper, U.S. Naval Research Lab. (United States); Kimihiro Susumu, Eunkeu Oh, Sotera Defense Solutions Inc. (United States); Igor L. Medintz, James B. Delehanty, U.S. Naval Research Lab. (United States)

Förster resonance energy transfer (FRET)-based assemblies currently comprise a significant portion of intracellularly based sensors. Although extremely useful, the fluorescent protein pairs typically utilized in such sensors are still plagued by many photophysical issues including significant direct acceptor excitation, small changes in FRET efficiency, and limited photostability. Luminescent semiconductor nanocrystals or quantum dots (QDs) are characterized by many unique optical properties including size-tunable photoluminescence, broad excitation profiles coupled to narrow emission profiles, and resistance to photobleaching, which can cumulatively overcome many of the issues associated with use of fluorescent protein FRET donors. Utilizing QDs for intracellular FRET-based sensing still requires significant development in many areas including materials optimization, bioconjugation, cellular delivery and assay design and implementation. We are currently developing several QD-based FRET sensors for various intracellular applications. These include sensors targeting intracellular proteolytic activity along with those based on theranostic nanodevices for monitoring drug release. The protease sensor is based on a unique design where an intracellularly expressed fluorescent acceptor protein substrate assembles onto a QD donor following microinjection, forming an active complex that can be monitored in live cells over time. In the theranostic configuration, the QD is conjugated to a carrier protein-drug analogue complex to visualize real-time intracellular release of the drug from its carrier in response to an external stimulus. The focus of this talk will be on the design, properties, photophysical characterization and cellular application of these sensor constructs.

10078-27, Session 7

Colloidal silicon quantum dots: from preparation to the modification of self-assembled monolayers for bioimaging and sensing applications

Xiaoyu Cheng, Temple Univ. (United States); Benjamin F. P. McVey, Andrew B. Robinson, Guillaume Longatte, Peter B. O'Mara, Vincent T. G. Tan, Pall Thordarson, Richard D. Tilley, Katharina Gaus, Justin Gooding, The Univ. of New South Wales (Australia)

For a sensor to function in biological environments, the sensing approach should be non-invasive and materials used should be nontoxic. Fluorescence microscopy meets the first criteria, while quantum dots are ideal candidates to fulfill the second if nanoparticles are made with biocompatible materials. Here we merge the two concepts in the first proof-of-concept study of protease sensing with nontoxic silicon quantum dots (SiQDs). In this work, fabrication of the SiQDs FRET protease sensor was achieved with a step-wise manner by first preparing water in-dispersible, alkene passivated construct with hydrosilylation. Nanoparticles were then modified with thiol-ene 'click' chemistry to covalently attach substrate peptides onto the surface, and then acceptor dyes, allowing FRET to occur. Cleavage of the peptides with the target enzyme facilitated the removal of the dyes from the surface of SiQDs, with the concomitant decrease in the fluorescent signal due to FRET. Enzymatic responses were monitored with both intensity and fluorescence lifetime based methods, with the latter performed with far-field optical imaging by fluorescence lifetime imaging microscopy (FLIM). The combination of interfacial design and optical imaging presented in this work opens new opportunities for bio-applications using nontoxic silicon quantum dots, which is especially relevant in the context of intracellular sensing and imaging.

10078-28, Session 8

Functionalized nanoparticles and applications *(Invited Paper)*

Maria-Eleni Kyriazi, Univ. of Southampton (United Kingdom); Otto Muskens, Univ of Southampton (United Kingdom); Antonios G. Kanaras, Univ. of Southampton (United Kingdom)

In this work we will present our latest developments on the design of functional nanoparticles for targeted applications. We will particularly focus on nanoparticles coating with DNA and performing specific functions.

10078-29, Session 8

Fluorescence imaging of aptamere mediated delivery of thiolated gold nanoparticles to cells *(Invited Paper)*

Alf B. Mews, Marina Mutas, Lisa Prisner, Christian Strelow, Univ. Hamburg (Germany)

Small gold nanoparticles (AuNPs) with a thiol-functionalized surface ligands show strong fluorescence emission in the visible range and can be used for fluorescence labelling of biological structures. Toward this purpose both, the fluorescence mechanism and also the bindig of specifically labelled AuNPs are investigated. In the first part of the talk we will focus on the fluorescence mechanism of thiolated gold particles and color tunability by different kinds of surface modifications. We will show that especially the long fluorescence lifetime of more than 100 ns is beneficial for biological labelling experiments to discriminate the relatively short decaying auto fluorescence of the cells from the fluorescence of the AuNPs functionalized with Mercaptoundecanoic acid (MUA-AuNPs). In the second part we will show how the AuNPs can be functionalized with a specific aptamer (AIR-3a) to bind selectively to a certain receptor (IL-6R). The binding and uptake of the particles is then investigated by means of fluorescence-lifetime imaging microscopy (FLIM). To distinguish between bound and uptaken MUA-AuNPs we used cross-sectional FLIM scans of individual layers at different heights through the cells. With these scans we are able to image the whole cell with the bound/uptaken MUA-AuNPs based on their different lifetimes. The results show that the particles can clearly be resolved despite of the broad fluorescence autofluorescence both on the cell membrane as well as inside the cells.

10078-30, Session 8

Bio-orthogonal coupling on PEG-modified quantum dots

Naiqian Zhan, Goutam Palui, Hedi Mattoussi, Florida State Univ. (United States)

We have designed two sets of aldehyde- and azide-modified ligands; these ligands also present lipoic acid anchors and PEG hydrophilic moieties (LA-PEG-CHO and LA-PEG-azide). We combined this design with a photoligation strategy to prepare QDs with good control over the fraction of intact reactive groups per nanocrystal.

We first applied the extremely efficient hydrazone coupling ligation to react the QD with hydrozinopyridine, which produces a well-defined absorption feature at 354 nm ascribed to the hydrazone chromophore. We exploited this signature to measure the number of aldehyde groups per QD when the fraction of LA-PEG-CHO per nanocrystal was varied, by comparing the optical signature at 354 with the molar extinction coefficient of the chromophore. This allowed us to extract an estimate for the number of LA-PEG ligand per QDs for a few distinct size nanocrystals. We further complemented these findings with the use of NMR spectroscopy to estimate of the ligand density using well defined signatures of the terminal protons of the ligands, and found a good agreement between the two techniques. We then showed that bio-orthogonal reactions based on CLICK and hydrazone coupling can be achieved using QDs presenting a mixture of azide and CHO functions. We anticipate that this strategy could be applied other nanoparticles such as those of Au and metals and semiconductor nanocrystals.

10078-31, Session 8

Enhanced cytosolic internalization of nanoparticles mediated by an anti-microbial peptide

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As control over the growth, stabilization and functionalization of inorganic nanoparticles continue to advance, interest in integrating these materials with biological systems is also growing. Much attention has been directed towards identifying effective approaches to promote cytosolic internalization of the nanoparticles while avoiding endocytosis. We describe the use of a chemically synthesized anti-microbial peptide, SVS-1 peptide, as a vehicle that facilitates the non-endocytic uptake of luminescent quantum dots (QDs) inside live cells. The N-terminal cysteine residue of the peptide is attached to the hydrophilic functionalized QDs via covalent coupling. Epi-fluorescence and confocal microscopy images show homogeneous distribution of the QD-conjugates throughout the cytoplasm of cell cultures. Additionally, the QD staining does not show co-localization with transferrin labelled endosomes or DAPI stained nucleii. The QD uptake observed in the presence of physical and pharmacological endocytosis inhibitors further confirms the physical translocation of QDs through the cell membrane. Live cell imaging data collected from these experiments support these conclusions. Additionally, we use flow cytometry analysis to quantify the NP uptake in different cell lines with respect to the NP size, composition and incubation time.

10078-44, Session PSun

Semiconductor quantum dot supraparticles for single molecule biodetection

Fatimata Dembele, Alexandra Fragola, Vincent Loriette, Nicolas Lequeux, Thomas Pons, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (France)

Applications of nanotechnology to molecular diagnostics can potentially improve the simplicity and sensitivity of biomolecular detection. Semi-conductor nanocrystals or quantum dots (QDs) in particular demonstrate several properties that make them good candidates for biomolecular recognition. These nanocrystals present narrow size-tunable emission spectra and a broad excitation spectrum. In addition, they offer higher photostability and brigthness compared to conventional organic dyes. In the recent years QDs have demonstrated in many applications ranging from single molecule tracking to in vivo imaging and bio-detection. The objective of this study is to develop a new diagnostic tool based on fluorescent nanobeads containing quantum dots for a fast and single-molecule detection. Our strategy is to self-assemble QDs into clusters of a few hundreds of nanometers in diameter for an even brighter fluorescence and easily detectable analytical signal. Our work focuses on the formation of monodisperse QD-based supraparticles (SPs). In order to ensure the colloidal stability of these SPs, we chose to grow a silica shell on their surface. Biocompatibility is further assured by a new type of polymer-silica hybrid presenting a zwitterionic chain for water solubility and reactive functions for conjugation with biomolecules. Preliminary results have shown that we can integrate the SPs into a microfluidic system for fast and efficient single-particle counting.

10078-45, Session PSun

Multifunctional biocompatible doxorubicin-loaded fucoidan-capped gold nanoparticles for drug delivery, enhancement therapeutic efficacy, and photoacoustic image contrast

Van Phuc Nguyen, Pukyong National Univ. (Korea, Republic of); Sung Won Kim, Kosin Univ. (Korea, Republic of); Panchanathan Manivasagan, Junghwan Oh, Hyun Wook Kang, Pukyong National Univ. (Korea, Republic of)

Successful development of safe and effective nanoparticles for enhancing cancer treatment such as drug delivery, photothermal therapy and photoacoustic image contrast is challenging. Although gold nanoparticles (AuNPs) have the potential to perform such a role, the potential toxicity on the human body and low photostability limit their applications. This study reports a multifunctional biocompatible of AuNPs modified with doxorubicin-loaded fucoidan (Dox-Fu) to reduce toxicity, to increase the efficacy of the drug delivery, to enhance heating effect, and to improve photoacoustic image contrast for safe and effective treatment. The synthesized Dox-Fu-AuNPs, spherical shaped and average particles size of ~82 nm, was used for the entire experiments. A 532 nm laser system was utilized to illuminate on both tumor cells and animal tissue. A group of 18 rabbits with 110 mm3 tumor size was irradiated after injection of 100 μl Dox-Fu-AuNPs. Temperature development was recorded in real-time by using a digital thermal camera. Tumor growth was measured every day after treatment. The experiment results showed that tumor injected with Dox-Fu-AuNPs (300 μl, 300 μg/ml) was enhanced 17 oC after laser irradiation within 4 mins at the power density of 0.1221 W/cm2 in comparison with tumor treated with laser only. The tumor growth showed that the tumors completely disappeared with Dox-Fu-AuNPs -assisted laser after period of 14 days, but not in laser and control models. Photoacoustic image contrast was enhanced 2.43 fold with the assistance of Dox-Fu-AuNPs. The proposed Dox-Fu-AuNPs can serve as a new multifunctional agent for effective tumor theranostic applications.

10078-46, Session PSun

Highly reproducible gold nanoshell arrays for SERS-detected immunoassays

Weiping Qian, Jian Dong, Mingde Guo, Wei Xie, Man Wang, Ying Wang, Southeast Univ. (China)

To address the reproducibility of SERS substrates, monodispersed gold nanoshells (GNSs) were synthesized to self-assemble closely ordered-stack GNSs arrays with crystalline structure by drop boundary evaporation with controlled concentration, temperature and angle with the horizontal plane. The GNSs arrays have high SERS effects and excellent reproducibility. By conjugating capture antibody to the GNSs arrays and detection antibody to free GNSs, a surface-enhanced Raman scattering (SERS)-detected immunoassay was developed for immune-sensing in the range from 0.1 ng/mL to 1μg/mL. The GNSs arrays based on drop boundary evaporation have potential to be commercial devices and can be highly sensitive, reproducible.

10078-47, Session PSun

Polyelectrolyte capsules as a delivery system for the cells gene expression

Yana V. Tarakanchikova, Saratov State Univ. (Russian Federation) and Univ. of Oulu (Finland); Gleb B. Sukhorukov, Queen Mary, Univ. of London (United Kingdom); Alexey P. Popov, Ganna Reint, Ilya Skovorodkin, Igor Meglinski, Univ. of Oulu (Finland)

Polyelectrolyte microcapsules fabricated by layer-by-layer (LbL) coating of a sacrificial template followed by the decomposition of this template have attracted increased interest as novel entities for drug delivery and diagnostic purposes. The microcapsule structure comprises oppositely charged polyelectrolyte layers deposited on a charged sacrificial core. Two types of biocompatible and biodegradable polymers have been used to design polymeric capsules. Polyelectrolyte microcapsules have the advantages of mild preparation conditions, multifunctionality, and the capacity to encapsulate large amounts of material for cells application. Intracellular delivery of various types of oligonucleotides is a promising approach for diagnostic and therapeutic manipulation of cellular activity in a broad range of applications. In this paper we aim to assess the in vivo cellular uptake and delivery siRNA by polyelectrolyte microcapsules for green fluorescence protein (GFP) gene expression. This mechanism aims at drug delivery applications involving scheduled administration or time-controlled delivery before reaching the release site, where it has clear advantages over common carrier systems exhibiting release directly after suspension (??? ??? ??? ??????????). Our data pave the way for further in vivo research on polyelectrolyte microcapsules. Biocompatible polyelectrolyte microcapsules perfectly fit for delivery of some oligonucleotides with the potential to address critical challenges and create new opportunities in the general area of controlled release and, more specifically, the controlled or localized delivery of DNA or other nucleic acid based materials.

10078-50, Session PSun

Preparation and physical characterization of magnetite nanoparticles (MNP) with aminosilane shell

Maryam Youhannayee, Christoph Janiak, Robert Rabenalt, Peter Albers, Mathias Getzlaff, Heinrich-Heine-Univ. Düsseldorf (Germany)

Magnetite Nanoparticles are widely studied because of their fascinating application and also biocompatibility and low toxicity in different fields of biology and medicine. These nanoparticles covered with Aminosilane are perfect candidates for hyperthermia therapy due to a nearly perfect prevention of wash-out. In this contribution, the synthesis of magnetite nanoparticles by chemical coprecipitation is presented. In a first step, magnetite nanoparticles were prepared by coprecipitation of ????2+ and ????3+ with ammonium. Subsequently, magnetite nanoparticles were coated with a ligand shell consisting of Aminosilane. Different techniques were used for morphology and structure characterization. Dynamic light scattering technique was carried out to investigate the size distribution of nanoparticles in wet environment. TEM images also prove the formation of spherical MNP. For characterizing the crystal structure, X-ray diffraction was used. These measurements show that different parameters such as preparation speed have a significant influence on the size of nanoparticles.

10078-51, Session PSun

Supramolecular delivery of photoactivatable fluorophores in developing embryos

Yang Zhang, Sicheng Tang, Lorenzo Sansalone, Ek Raj Thapaliya, James D Baker, Francisco Raymo, Univ. of Miami (United States)

The identification of noninvasive strategies to monitor dynamics within living organisms in real time is essential to elucidate the fundamental factors governing a diversity of biological processes. This study demonstrates that the supramolecular delivery of photoactivatable fluorophores in Drosophila

melanogaster embryos allows the real-time tracking of translocating molecules. The supramolecular transport of the fluorophores inside self-assembling nanocarriers enables the detection of their emission upon injection into an embryo and the immediate visualization of the organism without the delays that are associated with the long maturation times of genetically-encoded probes. The photoactivation mechanism designed into the fluorophores permits the conversion of an emissive reactant into an emissive product with resolved fluorescence, under mild illumination conditions that are impossible to replicate with conventional switching schemes based on bleaching. Thus, the combination of supramolecular delivery and fluorescence photoactivation is a powerful tool to monitor dynamics in vivo and can evolve into a general chemical tool to track motion in biological specimens.

Scheme 1. Photochemical conversion of an emissive reactant into an emissive product with resolved fluorescence permits the acquisition of images before and after the switching event. The mild illumination conditions sufficient for photoswitching and the optimal photophysical properties of the interconvertible species allow the real-time visualization of dynamic events in living Drosophila melanogaster embryos.

10078-53, Session PSun

Probing the intracellular fate of supramolecular nanocarriers and their cargo with FRET schemes

Ek Raj Thapaliya, Univ. of Miami (United States); Colin Fowley, Bridgeen Callan, University of Ulster (United Kingdom); Sicheng Tang, Yang Zhang, Univ. of Miami (United States); John F Callan, University of Ulster (United Kingdom); Francisco M Raymo, Univ. of Miami (United States)

Two structural designs for the covalent integration of borondipyrromethene (BODIPY) chromophores within amphiphilic macromolecular constructs were envisaged based on free-radical polymerization. We systematically incorporated the various amounts of monomers to achieve bright and compact macromolecules. Indeed, their brightness can be obtained up to four times greater than that of water-soluble model BODIPY monomers. The number of fluorophores per polymer chain was estimated by nuclear magnetic resonance spectroscopy and gel permeation chromatography to range from 1.0 to 7.4. At concentration greater than 100 ?g/mL, these macromolecules assemble into nanoparticles with hydrophilic interior and hydrophilic surface. Their hydrodynamic diameter varies from 8 to 14 nm and is significantly smaller than that of most of the fluorescent polymeric nanoparticles reported in the literature so far. Furthermore, these macromolecules can also be attached covalently to secondary antibodies and their bright fluorescence exploited to image immunolabeled biological preparations.

10078-32, Session 9

Effects of biomolecules on the electrokinetics of colloidal nanoparticles in liquid suspension *(Invited Paper)*

Clyde Midelet, École Normale Supérieure de Rennes (France); Jun-Yang Lin, Sung Tsang, Chen-li Sun, National Taiwan Univ. (Taiwan); Johanna Midelet, Antonios G. Kanaras, Univ. of Southampton (United Kingdom); Bruno Le Pioufle, Ecole Normale Supérieure de Cachan (France); Olivier Français, Ecole Normale Supérieure de Cachan (France); Martinus H. V. Werts, École Normale Supérieure de Rennes (France)

Alternating electric fields can induce various types of motion in liquid suspensions of colloidal nanoparticles. These electrokinetic phenomena depend on the parameters of the electric field (frequency, amplitude, 3D topology), the particles (size, shape, composition) and the suspending liquid (polarizability, ionic strength, pH). In particular, the dielectrophoretic forces on submicron colloidal particles are dependent on the properties of the electric double layer (the “ion cloud”) around these particles. This dependence provides a mechanism for detecting and quantifying interactions between biomolecules and these nanoparticles, which can be combined with optical and spectroscopic measurements.

Here, we report on functionalized plasmonic nanoparticles that are tracked inside microfluidic systems by dark-field video-microscopy. A high-gradient AC electric field is set up using transparent micro-electrodes. Electrohydrodynamic motion of the entire fluid and dielectrophoretic trapping of individual particles can be analyzed quantitatively by numerical methods. By switching the electric field synchronously with the video acquisition, the effect of biomolecules on the electrokinetic trapping can be quantified. The electromicrofluidic devices allow also for rapid measurement of diffusion coefficients. Coupled with spectral selection of different populations of plasmonic nanoparticle assemblies in a single sample, this platform offers potential for the development of optofluidic biosensing schemes based on the non-equilibrium behaviour of colloidal nanoparticle suspensions.

10078-33, Session 9

Molecular detection using colloidal semiconductor nanoheterostructures within an integrated microfluidic device *(Invited Paper)*

Yin Thai Chan, National Univ. of Singapore (Singapore)

The in-vitro detection efficiency of molecular targets ubiquitously depends on sensitivity, speed, specificity and cost. The integration of large-scale integrated microfluidics and highly fluorescent colloidal semiconductor nanoheterostructures (CSNs) into a single device potentially allows for parallelized, automated processing of nanoliter sample volumes with low detection limits, thereby addressing many of the key parameters relevant to any detection scheme. This may be attributed in part to the many salient optical properties of CSNs, such as large action cross-section, resistance to photobleaching and flexible surface chemistry. On the other hand, large-scale integrated microfluidics incorporates different chambers each independently capable of performing a key processing step, ultimately allowing for sample-to-answer operation. In this talk, the working principles of such a diagnostics platform will be elaborated on, and both its utility and limitations will be described with respect to the detection of histidine decarboxylase in human white blood cell samples and the detection of tetanus toxoid in fetal bovine serum.

10078-34, Session 9

Two synthetic routes to obtain chiroptically active and water soluble quantum dots

Dominika Wawrzynczyk, Wroclaw Univ. of Science and Technology (Poland)

Colloidal quantum dots (QDs) are considered to be ones of the most promising labeling agents for bio-imaging, nanomedicine and sensing applications due to their high quantum yield, intense emission and proved resistance to photobleaching. Well established hot injection synthesis techniques allow to obtain monodisperse QDs of high quality optical properties, however, those nanostructures form stable colloidal solutions in toxic organic solvents such as chloroform. This is why, for any bio-related applications efficient synthesis and surface functionalization techniques,

which yield water soluble QDs based fluorescent markers, are sought. Additionally, the proper selection of QDs surface coating ligands can further widen their sensing capability.

In this spirit, zwitterionic chiral capping ligands, with proven molecular recognition capabilities: L- and D- Penicillamine were used as a surface stabilizing agents for CdS and CdSe QDs. In the first approach the low temperature, water based, synthesis of CdS QDs in the presence of selected chiral molecules yield nanostructures in the form of tetrapods. The L- and D- Penicillamine molecules were also used for post-synthetic surface functionalization of series of CdSe QDs with different sizes. Both techniques showed promising results regarding the long term colloidal stability of QDs in water solutions. Additionally, the influence of the chiral ligands presence on the QDs surfaces on their optical properties were investigated, including measurements of pico- and nano-second time scale dynamics, and wide wavelength range two-photon absorption cross-sections. Those results contributed to the understanding of the ligand induced chiro-optical activity observed in the band gap absorption region of the QDs.

10078-35, Session 10

Interaction of colloidal nanoparticles with cells *(Invited Paper)*

Wolfgang J. Parak, Philipps-Univ. Marburg (Germany) and CIC Biomagune (Spain)

What happens to inorganic nanoparticles (NPs), such as plasmonic gold or silver, superparamagnetic iron oxide, or fluorescent quantum dot NPs, after they have been administrated to an animal or a human being? The review discusses the integrity, biodistribution, and fate of NPs after in vivo administration. First the hybrid nature of the NPs is described, by conceptually dividing them into the inorganic NP core, an engineered surface coating around the core which comprises the ligand shell and optionally also bioconjugation, and into the corona of adsorbed biological molecules. It is shown that in vivo all of these three compounds may degrade individually and that each of them can drastically modify the life-cycle and biodistribution of the whole hetero-structure. The NPs thus may be disintegrated into different parts, of which biodistribution and fate would need to be analyzed individually. Multiple labelling and quantification strategies for such purpose will be discussed. All reviewed data indicate that in vivo NPs no longer should be considered as homogeneous entity, but should be seen as inorganic/organic/biological nano-hybrids with complex and intricately linked degradation pathways.

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10078-36, Session 10

An improved, non-functionalized route to plasmonic nanoparticle based cellular probing through osmolyte mediation

Soumik Siddhanta, Ishan Barman, Johns Hopkins Univ. (United States)

Engineering nanostructured probes for ultra-sensitive detection of specific molecular species, our research seeks to capture the complex changes in cells and tissues that can predict disease progression in an individual. While such nanoparticle-based platforms are rapidly gaining a foothold in cancer diagnostics, one of the most concerning factors is the vulnerability of cells to the interaction with functional nanoparticles thereby raising the specter of systemic toxicity. The nanoparticles end up damaging the cells and disrupting cellular functions thereby impeding their imaging aim. Furthermore, PEGylation, and similar routes, force a tradeoff between desired nanoparticle properties (recognition, uptake, and reduced toxicity) and sensitivity of plasmon-enhanced spectroscopic sensing methods, such as surface-enhanced Raman spectroscopy (SERS) where the proximal presence of noble metal NP and the organic molecule of interest is key.

In this work, we report a trehalose-mediated, non-surface functionalized route for cell-nanoparticle interactions that maintains cell viability while allowing selective interaction of the nanoparticle with the cell surface receptors and subsequent internalization. Through careful electron microscopy of nanoparticle-prostate cancer cells interactions, we elucidated that there exists a dynamic equilibrium between “free” cytosolic diffusion of the nanoparticles and endocytosis through vesicle formation – and trehalose tilts the scale in favor of the latter to mask the toxic effects of the nanoparticles. The precise molecular interpretation of this behavior was further probed through SERS, which directly points towards the protein stabilization properties of trehalose mediation during interaction of the nanoparticles with the plasma membrane components.

10078-37, Session 10

A semi-colloidal substrate for surface enhanced Raman scattering

Behzad Sardari, Meriç Özcan, Sabanci Univ. (Turkey)

Preparing an appropriate surface enhanced Raman scattering (SERS) substrate has been a central subject since the SERS was introduced in the mid-1970s. Over the years different kinds of SERS substrates were developed like the assembly of colloidal nanoparticles in two-dimensional and 3-dimensional configurations, patterned substrates fabricated by lithography or other nano-scale fabrication techniques. Although it is a time consuming process and appropriate for large scale production, synthesis of colloidal nanoparticles is straightforward. However potential aggregation of the nanoparticles over the substrate is a significant disadvantage of this method. On the other hand, patterning of the substrate provides means of adjusting the uniformity and the inter-particle distance assuring the uniformity in enhancement factor, and absence of particles aggregation problem. Also it is repeatable. However, it is costly and time consuming, and much more complicated process which require high-tech fabrication facilities.

Synthesis of nanoparticles by electrolysis, which is the result of reduction and oxidation in the anode and cathode respectively, became popular recently. This is a simple and economical method for synthesizing the nanoparticles compared to most of the other methods, and usually one needs a solution as an electrolyte that contains the ions of nanoparticles of interest.

In this work, we utilize the electrolysis effect to prepare a semi-colloidal substrate for SERS application in which the nanoparticles are created on the anode surface and they act as an active medium for SERS. We have studied different metals including Copper (Cu), Brass, Zinc (Zn), Silver (Ag) and Aluminum (Al) as the electrodes and compared the enhancement factor on the Raman signal intensity. In the experiments we measured more than four orders of magnitude enhancement on the Raman peaks of Rhodamine B. The proposed method has some key advantages: it is a very simple and low cost technique and also can be used in real time since it is a quite fast process.

10078-38, Session 11

Development of near infrared I-III-VI quantum dots for in vivo imaging applications *(Invited Paper)*

Thomas Pons, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (France)

Near infrared (NIR) emitting quantum dots based on copper indium chalcogenides present unique optical properties for in vivo fluorescence imaging. Here we present the synthesis of CuIn(S,Se)2/ZnS core/shell QDs with 30-50% quantum yield in the NIR range. These nanoprobes are solubilized in water using a block copolymer surface ligand composed of multiple binding groups for enhanced stability and zwitterionic groups for solubility and minimized nonspecific adsorption. They present limited toxicity compared to heavy metal-containing QDs. These versatile nanoprobes can be directly injected in the peritumoral region for sentinel lymph node imaging. We also demonstrate their vectorization with RGD peptides or their incorporation in folic acid-functionalized silica particles to target specific cancer cells. Their long fluorescence lifetime enables rejection of autofluorescence using time-gated detection. This considerably enhances the sensitivity of in vivo fluorescence imaging. These QDs have been used for long term labeling of cancer cells ex vivo. Following reinjection of these cells, time-gated detection enables in vivo imaging of these cancer cells in the blood stream at the single cell level. Finally, these QDs can be doped with paramagnetic manganese ions to provide multimodal contrast in both fluorescence and magnetic resonance imaging.

10078-40, Session 11

Observation of the death process of cancer cells killed through surface plasmon resonance of gold nanoring with optical coherence tomography

Shih-Yang Chen, National Taiwan Univ. (Taiwan); Yulu He, Xi'an Jiaotong Univ. (China) and National Taiwan Univ. (Taiwan); Cheng-Che Hsieh, Wei-Hsiang Hua, Meng Chun Low, National Taiwan Univ. (Taiwan); Meng-Tsan Tsai, Chang Gung Univ. (Taiwan); Yean-Woei Kiang, Chih-Chung Yang, National Taiwan Univ. (Taiwan)

The use of a high-resolution optical coherence tomography (OCT) system with the operation wavelength around 800 nm to scan SCC4 cancer cells under different laser illumination conditions is demonstrated. The cancer cells are incubated with Au nanorings (NRIs), which are linked with photosensitizer, AlPcS, for them to be up-taken by the cells. Two Au NRI samples of different geometries for inducing localized surface plasmon (LSP) resonance around 1310 and 1064 nm are used. Four different lasers are utilized for illuminating the cells under OCT scanning, including 1310-nm continuous (cw) laser, 1064-nm cw laser, 1064-nm femtosecond (fs) laser, and 660-nm cw laser. The 1310- and 1064-nm cw lasers mainly produce the photothermal effect through the LSP resonance of Au NRIs for damaging the observed cells. Besides the photothermal effect, the 1064-nm fs laser can produce strong two-photon absorption through the assistance of the LSP resonance of Au NRI for exciting AlPcS to effectively generate singlet oxygen and damage the observed cells. The 660-nm laser can excite AlPcS through single-photon absorption for generating singlet oxygen and damaging the observed cells. With the photothermal effect, the observed cells can be killed through the process of necrosis. Through the generation of singlet oxygen, the cell membrane can be preserved and the interior substances are solidified to become a hard body of strong scattering. In this situation, the cells are killed through the apoptosis process. Illuminated by the 660-nm cw laser, a process of interior substance escape is observed through high-speed OCT scanning.

10078-41, Session 11

Cancer cell death processes in combining photothermal and photodynamic effects through surface plasmon resonance of gold nanoring

Yulu He, Xi'an Jiaotong Univ. (China) and National Taiwan Univ. (Taiwan); Jian-He Yu, Jen-Hung Hsiao, Yi-Chou Tu, Meng Chun Low, Wei-Hsiang Hua, Cheng-Che Hsieh, Yean-Woei Kiang, Chih-Chung Yang, National Taiwan Univ. (Taiwan); Zhenxi Zhang, Xi'an Jiaotong Univ. (China)

In combining the photothermal and photodynamic effects for killing cancer cells through the localized surface plasmon resonance (LSP) of photosensitizer-linked Au nanorings (NRIs), which are up-taken by the cells, the cells can be killed via different processes, including necrosis and apoptosis. In particular, the dominating effect, either photothermal or photodynamic effect, for cancer cell killing leading to either necrosis or apoptosis process is an important issue to be understood for improving the therapy efficiency. In this paper, we demonstrate the study results in differentiating the necrosis and apoptosis processes of cell death under different laser illumination conditions. With the LSP resonance wavelength of the Au NRIs around 1064 nm, the illumination of a 1064-nm cw laser can mainly produce the photothermal effect. The illumination of a 1064-nm fs laser can lead to LSP resonance-assisted two-photon absorption of the photosensitizer (AlPcS) for generating singlet oxygen and hence the photodynamic effect, besides the photothermal effect. Also, the illumination of a 660-nm cw laser can result in single-photon absorption of the photosensitizer for generating singlet oxygen and the photodynamic effect. By comparing the necrosis and apoptosis distributions in dead cells between the cases of different laser illumination conditions, we can differentiate the cancer cell killing processes between the photothermal effect, photodynamic effect, and the mixed effect.

10078-52, Session 11

Gold-mediated drug delivery for improved outcome in chemotherapy

Celina Yang, Ryerson Univ. (Canada); Jamie Uertz, CytoViva, Inc. (United States); Devika B. Chithrani, Ryerson Univ. (Canada)

Nanoparticles can be used to overcome the side effects due to poor distribution of anticancer drugs. Among other NPs, colloidal gold nanoparticles (GNPs) offer the possibility of transporting major quantities of drugs due to their large surface-to volume ratio while confining anticancer drugs as closely as possible to their biological targets through passive and active targeting ensuring limited harmful systemic distribution. In this study, we chose bleomycin (BLM) as the anticancer drug since its therapeutic efficiency is severely limited because of its side effects. Bleomycin was conjugated to GNPs through a thiol bond. The effectiveness of the chemotherapeutic drug, bleomycin, is observed by visualizing DNA double strand breaks and calculating the survival fraction. The action of the drug is known to be in the nucleus and our experiments have shown GNPs in the nucleus along with bleomycin. Use of GNPs to deliver bleomycin increased the therapeutic efficacy of the drug. Having a better understanding of the interaction of GNPs and drugs will establish a more successful NP-based platform for combined therapeutic approach in cancer research since GNPs can be used as radiation dose enhancers.

10078-42, Session 12

Development of nanoparticle-based force sensors for mechano-medicine *(Invited Paper)*

Khalid Salaita, Emory Univ. (United States)

The cell-material interface is important to many fields ranging from regenerative medicine to biosensing and immunology. How the cell-substrate junction influences cell biology remains a mystery. This is due, in part, to the lack of molecular tools that allow one to image and manipulate mechanical aspects of this cellular interface. In this talk, I will describe the development of a set of fluorescent probes (mechanophores) to investigate the role of forces at this living/non-living interface. Force probes take advantage of FRET and NSET mechanisms to determine the extension of a molecular "spring" to quantify tension. Tension probes are modular and the spring element can be engineered using PEG polymers, oligonucleotides, and proteins. Employing colloidal nanoparticles will be shown to provide significant improvements in sensitivity. Fluorescence polarization spectroscopy and super-resolution imaging coupled provide the highest resolution maps of cell mechanics. In the final part of the talk, I will describe the application of these probes in the study of integrin receptor mechanotransduction and T cell receptor activation. We demonstrate mechano-pharmacology applications of these nanoparticle force probes. Importantly, I will show that receptor mechanics are highly heterogeneous and play a critical role in immune functions.

10078-43, Session 12

Highlighting cancer cells with macromolecular probes *(Invited Paper)*

Sicheng Tang, Yang Zhang, Ek Raj Thapaliya, Adrienne Brown, James Wilson, Francisco Raymo, Univ. of Miami (United States)

Our laboratory developed macromolecular probes for cancer detection with bright fluorescence and high contrast. Their structural design is based on the covalent attachment of signaling fluorophores and targeting ligands to the hydrophilic and hydrophobic side chains of a common polymer backbone. The resulting amphiphilic macromolecules assemble spontaneously into particles with nanoscaled dimensions in aqueous environments to bring multiple chromophores in close proximity without any detrimental effects on their photophysical properties. In fact, the brightness of the resulting nanostructured constructs is several orders of magnitude greater than that of a single isolated chromophoric component. Furthermore, the targeting ligands connected to these supramolecular assemblies can associate with complementary receptors expressed on the surface of target cells to allow their detection with fluorescence measurements. Indeed, imaging experiments with model cell lines demonstrate that our strategy permits the selective signaling of cancer cells with outstanding signal-to-noise ratios. Thus, the operating principles engineered into our macromolecular probes can ultimately evolve into a general protocol for the convenient detection of cancer with optical methods.

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10079-1, Session 1

Spatially selective depleting tumor-associated negative regulatory T-cells with near infrared photoimmunotherapy (NIR-PIT): A new cancer immunotherapy *(Invited Paper)*

Hisataka Kobayashi, National Cancer Institute (United States)

Near infrared photoimmunotherapy (NIR-PIT) is a new type of molecularly-targeted photo-therapy based on conjugating a near infrared silica-phthalocyanine dye, IR700, to a monoclonal antibody (MAb) targeting target-specific cell-surface molecules. When exposed to NIR light, the conjugate rapidly induces a highly-selective cell death only in receptor-positive, MAb-IR700-bound cells. Current immunotherapies for cancer seek to modulate the balance among different immune cell populations, thereby promoting anti-tumor immune responses. However, because these are systemic therapies, they often cause treatment-limiting autoimmune adverse effects. It would be ideal to manipulate the balance between suppressor and effector cells within the tumor without disturbing homeostasis elsewhere in the body. CD4+CD25+Foxp3+ regulatory T cells (Tregs) are well-known immune-suppressor cells that play a key role in tumor immuno-evasion and have been the target of systemic immunotherapies. We used CD25-targeted NIR-PIT to selectively deplete Tregs, thus activating CD8+ T and NK cells and restoring local anti-tumor immunity. This not only resulted in regression of the treated tumor but also induced responses in separate untreated tumors of the same cell-line derivation. We conclude that CD25-targeted NIR-PIT causes spatially selective depletion of Tregs, thereby providing an alternative approach to cancer immunotherapy that can treat not only local tumors but also distant metastatic tumors.

10079-2, Session 1

The next generation of PhotochemCAD: Diverse software modules and databases including a simulations workbench for multicolor flow cytometry *(Invited Paper)*

Masahiko Taniguchi, Hai Du, Jonathan S. Lindsey, North Carolina State Univ. (United States)

PhotochemCAD is a software program and accompanying spectral database for use in photochemistry and photobiology. The development of PhotochemCAD was motivated by the desire to have at one's fingertips spectral data (absorption spectra with molar absorption coefficients, emission spectra with quantum yields, references to the original photochemical literature) for a wide variety of representative compounds and the ability to perform photochemically relevant calculations using such spectral data. PhotochemCAD 0 (1989), 1 (1998) and 2 (2005) have enabled calculations (Förster energy transfer, oscillator strength, fluorescence lifetime, multicomponent analysis, blackbody radiator, transmission) and simulations (energy transfer, artificial spectrum creation based on Lorentzian and Gaussian distributions) with an original spectral database of ~150 compounds.

PhotochemCAD 3 adds the following features: (1) expanded spectral databases, (2) multiple database handling capabilities, (3) a simulation workbench for flow cytometry, and (4) interface upgrades. Over 1000 new spectral data have been incorporated for ~400 commercial fluorophores (cyanine dyes, BODIPYs, Q-dot, Alexa Fluor, fluorescence proteins, etc.) and ~650 tetrapyrrole macrocycles (porphyrins, chlorins, bacteriochlorins, phthalocyanines). The spectral data in the PhotochemCAD database can be exported to other programs, and user-recorded spectral data are easily imported.

The availability of this program and database is anticipated to continue to support the design and analysis of a wide variety of photochemical systems in fields ranging from photonics to biomedical sciences. PhotochemCAD 3 will be available in due course for free downloading at http://www.photochemcad.com

10079-3, Session 1

Long lifetime near-infrared-emitting quantum dots for time-gated in vivo imaging of rare circulating cells

Alexandra Fragola, LPEM ESPCI (France) and Ctr. National de la Recherche Scientifique (France) and Univ. Pierre et Marie Curie (France); Sophie Bouccara, Institut Pasteur (France); Sophie Pezet, Lab. de Neurobiologie, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (France); Nicolas Lequeux, Vincent Loriette, Thomas Pons, LPEM ESPCI (France) and Ctr. National de la Recherche Scientifique (France) and Univ. Pierre et Marie Curie (France)

The in vivo detection of rare circulating cells using non invasive fluorescence imaging would provide a key tool to study migration of eg. tumoral or immunological cells. Fluorescence detection is however currently limited by a lack of contrast between the small emission of isolated, fast circulating cells and the strong autofluorescence background of the surrounding tissues. We present the development of near infrared emitting quantum dots (NIR-QDs) with long fluorescence lifetime for sensitive time-gated in vivo imaging of circulating cells. These QDs are composed of low toxicity ZnCuInSe/ZnS materials and made biocompatible using a novel multidentate imidazole zwitterionic block copolymer, ensuring their long term intracellular stability. Cells of interest can thus be labeled ex vivo with QDs, injected intravenously and imaged in the near infrared range. Excitation using a pulsed laser coupled to time-gated detection enables the efficient rejection of short lifetime (≈ ns) autofluorescence background and detection of long lifetime (≈ 150 ns) fluorescence from QD-labeled cells. We demonstrate efficient in vivo imaging of single fast-flowing cells, which opens opportunities for future biological studies.

[1] M. Tasso et al, "Sulfobetaine-Vinylimidazole block copolymers: a robust quantum dot surface chemistry expanding bioimaging's horizons", ACS Nano, 9(11), 2015

[2] S. Bouccara et al, "Time-gated cell imaging using long lifetime near-infrared-emitting quantum dots for autofluorescence rejection", J Biomed Optc, 19(5), 2014

10079-4, Session 1

Folic acid-conjugated graphene oxides as dual-function agent for photothermal therapy and imaging

Seung Won Jun, Soo Kyung Chun, Junyoung Kwon, Hyun

Ah Lee, Jaebeom Lee, Dae Youn Hwang, Chang-Seok Kim, Pusan National Univ. (Korea, Republic of)

Recently, folic acid-conjugated graphene oxides (FA-GOs) have received attention due to the cancer targeted photothermal therapy. FA-GOs have been employed to Near-infrared photothermal therapy (NIR PTT) due to their strong absorbance at the 800 nm wavelength region. NIR PTT based on nanoparticles provides a highly localized and low power/energy cancer therapy with minor undesired side effects to normal tissues. It has been known that most of these researches are focused on the NIR absorbed photothermal effect of FA-GOs.

On the other hand, it is also reported that graphene oxides (GOs) themselves have a three-photon induced strong photoluminescence (PL) at the 1200 nm wavelength region. In this work, folic acid (FA), a targeting molecule to cancer cells, was conjugated to GOs via covalent amide bond. Obtained FA-GOs were proved to be probe for NIR PTT and dye for three photon microscopy (3PM) imaging. Our research revealed the three-photon induced fluorescence from FA-GOs by using a custom-built 3PM to obtain the three-photon induced fluorescence image from FA-GOs. The 3PM is promising tool for cancer detection and imaging owing to the deep imaging depth, low background signal, eliminated autofluorescence of sample, reduced photobleaching, and reduced phototoxicity.

To demonstrate the capacity of FA-GOs for dual-function agent for NIR PTT and imaging, we used human breast cancer cell lines (MCF7) and normal human mammary epithelial cell line (MCF-10A). Our results suggest that the FA-GOs can be used dual-function agent for localized heating for NIR PTT and optical contrast probe for 3PM.

10079-5, Session 1

Nonlinear SWIR imaging

Vladislav V. Yakovlev, Texas A&M Univ. (United States)

Short wavelength infrared (SWIR) part of the spectrum offers several distinct advantages for biomedical imaging. In this report, we discuss applications of short-pulse SWIR radiation for nonlinear optical imaging. In particular, we utilized second- and third-harmonic generation microscopy of 1700-nm light to visualize collage structures and use a combination of 1200 nm and 1700 nm light for highly chemically selective coherent Raman excitation.

10079-6, Session 2

Photoacoustic imaging of hepatocellular carcinoma targeting gold nanoshells

Quan Zhou, Yan Chen, Univ. of Michigan (United States); Zhao Li, Peking Univ. People's Hospital (China); Juan Zhou, Xiyu Duan, Thomas D. Wang, Univ. of Michigan (United States)

Plasmonic gold nanoshell (GNS) probe penetrates into tumors for deep imaging, enables superior photoacoustic contrast. Glypican-3 (GPC3) specific peptide (Kd = 71 nM) conjugated gold nanoshell (?abs=770nm) was used to detect HCC xenograft tumors in mice with photoacoustic imaging. This targeting probe demonstrated tumor uptake after 1 hr and cleared in 12 hrs. Images at a mean (±SD) depth of 9.7±1.4 mm from 0 to 2.1 cm beneath the skin revealed increased PA signal from tumors. Highest tumor uptake and tumor to normal tissue ratio occurred at 2 hrs post injection (T/B = 4.45±0.22, n = 8). Molecular targeting GNS showed potential as a simple, effective and rapid technique for noninvasive in vivo monitoring HCC tumor growth and GPC3 expression.

10079-7, Session 2

Microscopic investigation of topically applied SERS NPs for Raman-encoded molecular imaging of fresh surgical specimens

Soyoung Kang, Yu Wang, Jonathan T. C. Liu, Univ. of Washington (United States)

Nanoparticles (NPs) bearing surface-conjugated targeting ligands are increasingly being explored for a variety of molecular imaging and diagnostic applications, such as intraoperative guidance of tumor resection. For example, we have previously shown that targeted SERS NPs that are topically applied on fresh tissues are able to rapidly target cell-surface protein biomarkers for the imaging of cancer. In particular, a dual-agent approach, in which an untargeted NP controls for the nonspecific behavior of a panel of targeted NPs, enables quantitative imaging of biomarker expression. However, given the complexities in the nonspecific accumulation, diffusion, and chemical binding of targeted NPs with cell-surface proteins in intact tissues, studies are needed to better understand these processes at the microscopic scale. Here, we stain fresh tissues with a dual-agent approach, and then perform fluorescence microscopy of frozen sections from these tissues, to study (at the microscopic level) the diffusion and binding of targeted and untargeted NPs (120-nm diameter) in fresh tissues. The method developed in this study – to quantify the penetration and binding of targeted and untargeted NPs as a function of depth at the microscopic level – will be valuable for optimizing topical staining conditions and informing the design of optimized NPs for the rapid molecular imaging of fresh tissue surfaces.

10079-8, Session 2

Enhanced esophageal tumor imaging with optical coherence tomography using gold nanoparticles

Chandra Jinata, Luoqin Yu, Naushad Hossain, Zhenbo Ren, Edmund Y. Lam, Marco C. Wong, Wing-Tat Chan, Hector K. Wang, Gus C. Chan, Kin-Tak Chan, Nikki P. Lee, Kenneth K. Y. Wong, The Univ. of Hong Kong (Hong Kong, China)

Early detection and treatment of esophageal cancer (EsC)-one of the deadliest cancers in Asia, can improve patients' survival rate. Optical coherence tomography (OCT), a non-invasive imaging modality, is a favourable tool to detect the presence of the tumor. Moreover, nanotechnology holds the promise of development of effective and safe cancer therapeutic-diagnostic (theranostic) agents, particularly gold nanoparticle (AuNP) due to its impressive biocompability and localized surface plasmon resonance (LSPR) characteristic. In this study, fully PEGylated gold nanorods (AuNRs) with LSPR peak at 1,300 nm were used as contrast agent for esophageal tumor imaging with OCT centred at 1,310 nm. KYSE450 cancer cells were firstly implanted subcutaneously to BALB/c nude mice and after three weeks of tumor growth, the 200 μg AuNRs suspension was injected intravenously to the tumor-bearing mice and OCT imaging was conducted after 48 hours of AuNRs injection. Compared to the control group which was injected by PBS, tumor OCT images from the AuNRs-treated group showed significant signal improvement by 48.3% ($p<0.000001$). Due to the limited targeting capacity of the current AuNRs, most of the injected AuNRs are accumulated in spleen, while only 1% (relative to spleen) resides inside the tumor. This proof-of-concept study emphasizes the encouraging direction towards EsC nanotheranostic agents development by further combining specific targeting ligand density strategy, laser-triggered drug release system, and tumor imaging contrast agent capability of the AuNP.

10079-9, Session 2

Silica passivated conjugated polymer nanoparticles for biological imaging applications

Struan Bourke, Laura Urbano, King's College London (United Kingdom); Antoni Olona, Ferran Valderrama, St. George's Univ. of London (United Kingdom); Lea Ann Dailey, Mark A. Green, King's College London (United Kingdom)

Colorectal and prostate cancers are major causes of cancer-related death, with early detection key to increased survival. However as symptoms occur during advanced stages and current diagnostic methods have limitations, there is a need for new probes that remain bright, are biocompatible and can be targeted. Conjugated polymer nanoparticles have shown great promise in biological imaging due to their unique optical properties. We have synthesised small, very bright, very photostable CN-PPV, nanoparticles encapsulated with poloxamer polymer and a thin silica shell that have shown good cellular uptake with limited cytotoxocity. These can be further functionalised for target specific binding.

10079-37, Session 3

The art of falling apart: exploiting nanomaterial disassembly for medicine and pharmacy *(Keynote Presentation)*

Adah Almutairi, Univ. of California, San Diego (United States)

Research in nanotechnology over the last two decades has enabled scientists and engineers to build constructs of shapes, sizes and properties previously unattainable. These efforts have been rewarded with numerous Nobel Prizes. Now is the time to also focus on controlled disassembly of nanomaterial constructs to access properties and enable technologies useful in overcoming medical and pharmaceutical challenges. This presentation will cover two new classes of materials developed. Their usefulness to a number of medical challenges such as gene delivery and inflammatory diseases will be highlighted.

10079-11, Session 4

Nanodiamond preparation and surface characterization for biological applications

Ben Woodhams, Cavendish Lab., Univ. of Cambridge (United Kingdom) and Cancer Research UK Cambridge Institute, Univ. of Cambridge (United Kingdom); Helena Knowles, Dhiren Kara, Mete Atatüre, Cavendish Lab., Univ. of Cambridge (United Kingdom); Sarah E. Bohndiek, Cavendish Lab., Univ. of Cambridge (United Kingdom) and Cancer Research UK Cambridge Institute, Univ. of Cambridge (United Kingdom)

Nanodiamonds contain stable fluorescent emitters and hence can be used for molecular fluorescence imaging and precision sensing of electromagnetic fields. The physical properties of these emitters together with their low reported cytotoxicity make them attractive for biological imaging applications. The controlled application of nanodiamonds for cellular imaging requires detailed understanding of surface chemistry, size ranges and aggregation, as these can all influence cellular biocompatibility.

We compared these characteristics for graphitic and oxidized nanodiamonds. Oxidation is generally used for surface functionalization, and was achieved by 450°C heating in air for one hour then confirmed via Raman and Infrared spectroscopies. Size ranges and aggregation were assessed using Atomic Force Microscopy and Dynamic Light Scattering. Biocompatibility in breast cancer cell lines was measured using assays of uptake, proliferation and oxidative stress in phase and fluorescence microscopy.

Heating at 450°C reduced the Raman signal of graphitic carbon (1575 cm-1) as compared to that of diamond (1332 cm-1). The fraction of nanodiamonds with diameter <10 nm was reduced from 44±7% in the unheated sample to 26±3% after oxidation, partially decreasing the fraction of nanodiamonds that are expected to be free in the cytosol. Graphitic nanodiamonds formed aggregates in water, with a particle size of 210±30nm at a concentration of 10?g/ml. We then applied the 1?g/ml graphitic and oxidized nanodiamonds to cells in culture, to determine biocompatibility and found no significant change in the proliferation rate (+1±2% and -8±8% respectively). Nanodiamonds may therefore be suitable for development as a novel transformative tool in the life sciences.

10079-12, Session 4

Photoswitchable dye-nanoparticle probes with photothermal switching of light-dark states and colors

Walter Harrington, Univ. of Arkansas for Medical Sciences (United States); Mwafaq R. Haji, Univ. of Arkansas at Little Rock (United States); Ekaterina I. Galanzha, Dmitry A. Nedosekin, Univ. of Arkansas for Medical Sciences (United States); Zeid A. Nima, Fumiya Watanabe, Anindya Ghosh, Alexandru S. Biris, Univ. of Arkansas at Little Rock (United States); Vladimir P. Zharov, Univ. of Arkansas for Medical Sciences (United States)

In recent years, there has been a heightened interest in photoswitchable proteins for their potential to track of biomolecules, cellular organelles and cells as a whole. The ability to remotely switch the fluorescence of these proteins makes it possible to track even singular cells in the bloodstream, as has been shown by us using cells genetically engineered to produce photoswitchable proteins. There are limitations, however, such as the need for high energy UV light to switch the proteins, which brings about phototoxicity concerns and has low penetration in biotissue. To overcome these limitations, here we present of a novel strategy for developing photoswitchable probes of which absorption (i.e., not fluorescence) is responsive to near-infrared light, leading to change absorption spectra (color), or light—dark absorption states. The proof-of-principle was demonstrated using thermochromic dyes mixed with magnetic nanoparticles. We are able to show that the nanoparticles effectively absorb near-infrared NIR light, converting it to heat which in turn causes color shift of the thermo-sensitive dyes. Specifically, we show that the absorption spectrum of these probes can be switched using 805 nm laser both in bulk solution and at the single bead level. In contrast to conventional fluorescence (i.e., emission) photoswitching, the laser-induced the absorption change of these probes using photothermal phenomena makes them a good candidate for photoacoustic detection. We present this strategy as a proof-of-principle stepping stone for developing multifunctional photoswitchable probes that can be used for photoacoustic detection of circulating tumor and bacterial cells in deep vessels.

10079-13, Session 5

Novel molecular-based fluorescent nanoparticles for three-photon excited microscopy at 1700 nm

Charles-Henri Hage, XLIM Institut de Recherche, Univ. de Limoges (France) and Ctr. National de la Recherche

Scientifique (France); Patrick Cadroas, XLIM Institut de Recherche, Univ. de Limoges (France) and NOVAE (France) and Ctr. National de la Recherche Scientifique (France); Jonathan Daniel, Paolo Pagano, Christiano Mastrodonato, Institut des Sciences Moléculaires, Univ. Bordeaux 1 (France) and Ctr. National de la Recherche Scientifique (France); Dmitry A. Gaponov, NOVAE (France); Raphael Jauberteau, XLIM Institut de Recherche, Univ. de Limoges (France) and Ctr. National de la Recherche Scientifique (France); Pierre Leclerc, XLIM Institut de Recherche, Univ. de Limoges (France) and Ctr. National de la Recherche Scientifique (France); Marc Fabert, Julien Brevier, Rodney P. O'Connor, Sylvia M. Bardet-Coste, Frederic Louradour, Sébastien Février, XLIM Institut de Recherche, Univ. de Limoges (France) and Ctr. National de la Recherche Scientifique (France); Mireille H. Blanchard-Desce, Institut des Sciences Moléculaires, Univ. Bordeaux 1 (France) and Ctr. National de la Recherche Scientifique (France)

Recent studies showed that the excitation spectral window lying between 1.6 and 1.8 ?m is optimal for in-depth three-photon microscopy of intact tissues due to the reduced scattering and absorption in this wavelength range. Hence, millimeter penetration depth imaging in a living mouse brain has been demonstrated, demonstrating a major potential for neurosciences.

Further improvements of this approach, towards much higher imaging frame rates (up to 15-20 s/frame in previous achievements) requires the development of advanced molecular optical probes specifically designed for three-photon excited fluorescence in the 1.6 -1.8 ?m spectral range.

In order to achieve large three-photon brightness at 1700 nm, novel molecular-based fluorescent nanoparticles which combine strong absorption in the green-yellow region, remarkable stability and photostability in aqueous and biological conditions have been designed using a bottom-up route. Due to the multipolar nature of the dedicated dyes subunits, these nanoparticles show large nonlinear absorption in the NIR region.

These new dyes have been experimentally characterized through the measurement of their three-photon action cross-section, fluorescence spectra and lifetimes using a monolithically integrated high repetition rate all-fiber femtosecond laser based on soliton self-frequency shift providing 9 nJ, 75 fs pulses at 1700 nm. The main result is that their brightness could be several orders of magnitude larger than the one of Texas Red in the 1700 nm excitation window.

Ongoing experiments involving the use of these new dyes for in vivo cerebral angiography on a mouse model will be presented and the route towards three-photon endomicroscopy will be discussed.

10079-14, Session 5

Novel microfabrication stage allowing for one-photon and multi-photon light assisted molecular immobilization and for multi-photon microscope

Odete Sofia Lopes Gonçalves, Steffen Bjorn Petersen, Aalborg Univ. (Denmark); Scott Snider, Ruben Zadoyan, Newport Corporation, Technology & Applications Center (United States); Quoc-Thang Nguyen, NeurAccel Biosciences (United States); Henrik Vorum, Aalborg Univ. Hospital (Denmark); Maria Teresa Neves-Petersen, Aalborg Univ. (Denmark)

Light Assisted Molecular Immobilization (LAMI) results in spatially oriented and localized covalent coupling of biomolecules onto thiol reactive surfaces. LAMI is possible due to a conserved structural motif in proteins: the spatial proximity between aromatic residues and disulfide bridges. When aromatic residues are excited with UV light (275-295nm), disulphide bridges are disrupted and free thiol groups are formed that can bind covalently to a surface.

Immobilization is achieved in a microfabrication stage coupled to a femtosecond laser, through one- or multi-photon excitation lasting a few miliseconds. Through one-photon excitation, the fundamental output is tripled leading to a UV output which is focused onto the sample. The focused beam is moved in desired patterns, being the molecules immobilized according to such patterns and reaching submicrometer spatial resolution.

We have achieved successful immobilization of proteins such as Bovine Serum Albumin, Lysozyme, C-Reactive protein and of molecular beacons designed to recognized miRNA cancer markers. Molecular beacons were coupled to a small peptide containing a disulfide bridge/aromatic residue. This technology has great potential for biomedical, nanotechnology and therapeutic applications.

10079-15, Session 5

Rhodamine-based activatable protease probes for fluorescence and photoacoustic cancer imaging

Ryu J. Iwatate, The Univ. of Tokyo (Japan); Mako Kamiya, The Univ. of Tokyo (Japan) and Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency (Japan); Takeshi Hirasawa, Miya Ishihara, National Defense Medical College (Japan); Yasuteru Urano, The Univ. of Tokyo (Japan) and Japan Science and Technology Agency (Japan) and Japan Agency for Medical Research and Development (Japan)

To achieve rapid and sensitive detection of cancer during surgery, we have developed activatable fluorescent probes targeting proteases that are overexpressed in various types of cancer [1]. These include gGlu-HMRG, a fluorescent probe for ?-glutamyl transpeptidase (GGT) based on the hydroxymethyl rhodamine green (HMRG) scaffold [2], which has achieved in vivo tumor detection with high tumor-to-background ratio. However, to visualize altered activities of multiple enzymes in cancer sites, other scaffolds with distinct fluorescence properties from those of HMRG are needed. Here, we introduce a strategy to rationally develop activatable protease probes with desired properties.

By synthesizing asymmetrically modified rhodamines, we have enabled the production of scaffolds with desired wavelength and equilibrium constant of intramolecular spirocyclization pKcycl (the pH at which the absorbance of the compound decreases to a half of the maximum absorbance as a result of spirocyclization). As a proof of concept, a probe targeting GGT, gGlu-HMJCR, was developed based on a novel scaffold with desired properties; bright, red-shifted fluorescence and marked fluorescence activation upon reaction with the target enzymes [3]. For probes with even longer wavelengths, we have also applied this design scheme to Si-rhodamines.

Scaffolds with low quantum yield can also be applied to photoacoustic imaging, potentially useful for imaging deeper tissues.

[1] M. Sakabe et al., J. Am. Chem. Soc. 2013, 135 (1), 409-14.

[2] Y. Urano et al., Sci. Transl. Med. 2011, 3, 110ra119.

[3] R. J. Iwatate et al., Chem. - A Eur. J. 2016, 22 (5), 1696–1703.

10079-16, Session 6

Protein tethering enables rapid and label-free SERS platform for screening drugs of abuse

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A quick, cost-effective method for detection of drugs of abuse in biological fluids would be of great value in healthcare, law enforcement, and home testing applications. The alarming rise in narcotics abuse has led to considerable focus on developing potent and versatile analytical tools that can address this societal problem. While laboratory testing plays a key role in the current detection of drug misuse and the evaluation of patients with drug induced intoxication, these typically require expensive reagents and trained personnel, and may take hours to complete. Thus, a significant unmet need is to engineer a facile method that can rapidly detect drugs with little sample preparation, especially the bound fraction that is typically dominant in the blood stream.

Here we report an approach that combines the exquisite sensitivity of surface enhanced Raman spectroscopy (SERS) and a facile protein tethering mechanism to reliably detect four different classes of drugs, barbiturate, benzodiazepine, amphetamine and benzoylecgonine. The proposed approach harnesses the reliable and specific attachment of proteins to both drugs and nanoparticle to facilitate the enhancement of spectral markers that are sensitive to the presence of the drugs. In conjunction with chemometric tools, we have shown the ability to quantify these drugs lower than levels achievable by existing clinical immunoassays. Through molecular docking simulations, we also probe the mechanistic underpinnings of the protein tethering approach, opening the door to detection of a broad class of narcotics in biological fluids within a few minutes as well as for groundwater analysis and toxin detection.

10079-17, Session 6

Modeling of transdermal fluorescence measurements from first-in-human clinical trials for renal function determination using fluorescent tracer agent MB-102

Kimberly M. Shultz, Triple Ring Technologies, Inc. (United States); Martin P. Debreczeny, Richard B. Dorshow, MediBeacon, LLC (United States); Jennifer E. Keating, Kate L. Bechtel, Triple Ring Technologies, Inc. (United States)

The fluorescent tracer agent, 2,5-bis[N-(1-carboxy-2-hydroxy)]carbamoyl-3,6-diaminopyrazine, designated MB-102, is cleared from the body solely by the kidneys. A prototype noninvasive fluorescence detection device has been developed for monitoring transdermal fluorescence after bolus intravenous injection of MB-102 in order to measure kidney function. A mathematical model of the detected fluorescence signal was created for evaluation of observed variations in agent kinetics across body locations and for analysis of candidate instrument geometries. The model comprises pharmacokinetics of agent distribution within body compartments, local diffusion of the agent within the skin, Monte Carlo photon transport through tissue, and ray tracing of the instrument optics. Model validation was performed by estimating the concentration of MB-102 in the interrogated sample from both in vitro and in vivo experiments. Data from eight human subjects with normal renal function and a range of skin colors shows good agreement with simulated data. Body site dependence of equilibration kinetics was explored using the model to find the local vascular-to-interstitial diffusion time constant, blood volume fraction, and interstitial volume fraction. Finally, candidate instrument geometries were evaluated using the model. While an increase in source-detector separation was found to increase sensitivity to tissue optical properties, it reduced the relative intensity of the background signal with minimal effect on the measured equilibration kinetics.

10079-18, Session 6

Transcutaneous measurement of glomerular filtration rate in conscious laboratory animals: State of the art and future perspectives

Jochen Friedemann, Daniel Schock-Kusch, Yury Shulhevich, MediBeacon GmbH (Germany)

Transcutaneous measurement of glomerular filtration rate (tGFR) is now frequently used in preclinical in vivo animal studies. tGFR allows consecutive measurements on the same animal, including multiple measurements on a daily basis. A description of the measurement device and its many applications, along with examples from the recent literature will be given. We will highlight the fields of interest in which the system is used and give an overview about its performance versus endogenous and other exogenous methods of GFR measurement.

A special focus will be put on the precision of tGFR compared to standard measurements employed in the research setting. A novel kinetic model for the description of transcutaneously measured excretion kinetics of the fluorescent GFR tracer FITC-Sinistrin was recently described. Using this new kinetic model (designated tGFR3cp.b.m), tGFR measurements in the rat model reached comparable precision as GFR measurements assessed using a gold standard technique based on constant infusion (cGFR). The precision of tGFR assessment when using tGFR3cp.b.m, coupled with the capability of consecutive GFR measurements in the same animal over a long time span should enhance the quality of, and reduce the cost of, preclinical assessment of renal function in pharmaceutical research.

10079-19, Session 6

Development and clinical trial results of a prototype device for trans-cutaneous monitoring of kidney function

Martin P. Debreczeny, Richard B. Dorshow, MediBeacon, LLC (United States)

A prototype medical device for trans-cutaneous monitoring of kidney function has been developed, validated, and used in a clinical trial on 16 healthy subjects having a wide range of skin color types. The fluorescent tracer agent MB-102 was administered intravenously as a bolus that was varied between 0.5 and 4 umole/kg subject weight. The tracer agent was tracked as a function of time in plasma by blood sampling and trans-cutaneously at four body sites (sternum, forehead, arm, and side) simultaneously. Excitation was performed with a very low level of LED light at 450 nm (<50 uW/cm2), and fluorescence emission was synchronously detected at 570 nm. With adjustment of detection gain between subjects, no skin color dependence was observed of the signal-to-noise ratio (SNR) of the trans-cutaneous measurements. The primary source of measurement noise appeared to be subject motion, likely due to variations in blood content at the skin measurement site. A typical two-compartment pharmacokinetic dependence was observed with equilibration of the fluorescent agent between the vascular space into which it was injected and the extracellular space into which it subsequently diffused. Variation of this equilibration time was observed across body sites, with the sternum providing the shortest and most consistent equilibration. After equilibration, the terminal fluorescence time dependence at the sternum site was found to be highly correlated with tracer agent concentration time dependence sampled from the blood plasma.

10079-20, Session 6

Dynamics of singlet oxygen generation in aqueous solution of Radachlorin photosensitizer

Oleg S. Vasyutinskii, Dina M. Beltukova, Irina V. Semenova, Andrey G. Smolin, Viktor P. Belik, Idris M. Gadzhiev, Ioffe Institute (Russian Federation)

The thorough in vitro evaluation of photophysical properties of photosensitizers applied in photodynamic therapy is a prerequisite of their efficient usage in medical practice. In this paper we present experimental and theoretical study of in vitro dynamics of singlet oxygen (SO) generation in aqueous solution of the photosensitizer Radachlorin (PS). The experiments have been carried out under the steady-state and pulse laser excitation conditions. The entire spectrum of the solute luminescence in the wavelength range 660-1300 nm has been recorded and analyzed as function of time and the PS concentration. The PS photobleaching which is one of the important factors affecting the photodynamic therapy efficiency has been studied among other photophysical parameters. The results obtained have been interpreted on the basis of solution of a set of rate equations describing excitation and degradation of the PS and SO molecules. As shown, in the conditions of our experiment the PS photobleaching occured mainly via a non-SO-mediated mechanism dealing with electron-transfer chemical reaction between the excited triplet state PS molecules and the ground state oxygen molecules. Bleaching rate coefficient and other photophysical parameters characterizing singlet oxygen generation and degradation have been determined from experiment. Using picosecond pulse laser excitation and the time correlation single photon counting (TCSPC) technique for luminescence detection we demonstrated almost complete separation of the SO phosphorescence signal at 1270 nm from the PS luminescence signal. The results reported can be utilized for providing optimal photosensitizer concentration and excitation light intensity for singlet oxygen generation in solutions and cells.

10079-23, Session PMon

Aggregation-induced near-infrared (700-900nm) fluorophores of terrylenediimide-tetraphenylethene dyads

Ming-Qiang Zhu, Huazhong Univ. of Science and Technology (China)

We design and synthesize terrylenediimide–tetraphenylethene dyads, which exhibit featured aggregation-induced near-infrared fluorescence (700-900 nm) with a maximum emission wavelength of up to 800 nm.

10079-24, Session PMon

The dependence of the mechanical properties on sizes of micropore of sorbents

Anna Kolesnikova, Saratov State Univ. (Russian Federation)

The porous carbon materials (sorbents) humanity uses for many centuries. Currently, the main directions of use of carbon sorbents are related to the technological processes of the adsorption treatment, separation and concentration in gaseous and liquid media. Carbon sorbents are used in medicine and pharmaceutics. Carbon hemosorbents clean clean the blood outside the body of patients. Chelation are taken into the body to cleanse it from harmful substances and microbes. Carbon sorbents exist in various forms: powder, granules, blocks of different shapes and sizes, films et. al.

Among the large variety of porous structures greatest interest are the porous carbon structures having a density of 1.4 g/cm3. This interest is due to a well-defined synthesis technology of this material. The mechanical stability of the sorbent is its important property. Sorbent with high mechanical properties can be used for filtration of heavy particles moving at high speeds. Therefore the aim of this work is to study the mechanical strength of the porous carbon structures with a density of 1.4g / cm3 with different pore sizes. It was investigated the influence of pore size and form of the sorbent on its adsorption capacity. The study of the mechanical properties of porous structures was based on the unit cell using molecular mechanical method REBO, and taking into account the periodic conditions in three areas of the porous structure.

10079-25, Session PMon

Management the strength properties of carbon composites

Anna Kolesnikova, Margarita Mazepa, Saratov State Univ. (Russian Federation)

Actual problems in medicine is to find new materials to create sorbents on base a composite material with ordered pores and high mechanical properties. A new direction in the development of such materials is related to the synthesis and study of properties of composite carbon nanostructures (CCNS) based on carbon nanotubes and graphene. Investigation the deformation of the composite under tension along the graphene sheet is carried out for the first time in this work by molecular mechanical method based on a reactive empirical bond order (REBO). This deformation direction of the composite is selected in accordance with the fact that deformation of graphene in the longitudinal direction and deformation of nanotubes in the transverse direction will occur during the filtration of microorganisms.

Series of numerical of experiments was carried out on the composite cell with periodic boundary conditions. Periodic boundary conditions were set in the plane graphene sheet. Setting of periodic conditions only in two directions was conditioned the fact that infinitely long composites are observed in the experiment only in the plane of the graphene sheet. The diagram can construct in accordance with the dependence of the force which must be applied for stretching the composite 1%, on the geometric parameters of the composite. You can determine on this diagram the minimum size of the composite to be used as nanofilter, knowing the power of the action of microorganisms on the composite.

10079-26, Session PMon

BODIPY-based visible-light activatable caging groups with improved single-photon uncaging efficiency

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Photoremovable protective groups, or caging groups, enable us to regulate the activities of bioactive molecules in living cells upon photoirradiation. Nevertheless, requirement of UV light for activating caging group is a significant limitation due to its cell toxicity and its poor tissue penetration. Recently, our group developed a novel caging group based on BODIPY, a green fluorophore (N. Umeda, et al., ACS. Chem. Biol. 2014). BODIPY caging group can be activated by 500 nm visible light, but the photoreaction efficiency is lower than conventional caging groups. In this study, we aimed at improving photoreaction efficiency of the BODIPY caging group. We

hypothesized that the charge separation state after photo-induced electron transfer (PeT) would be the key intermediate to trigger deprotection of the BODIPY caging group, and synthesized several derivatives with different BODIPY cores (different reduction potential) and different leaving groups (different oxidation potential) to change the driving force of PeT. Examination of the photochemical properties of the synthesized BODIPY derivatives revealed that the uncaging quantum yields were dependent not only on the driving force for PeT process, but also on the substituent groups on BODIPY cores. One of these derivatives exhibited an improved uncaging quantum yield compared to the previously reported one (2.3 times higher). Moreover, the uncaging quantum yields dramatically increased in less polar solvents. Especially in hexane, it reached over 1%, whose uncaging efficiency is higher than those of several UV light-activatable caging groups.

10079-27, Session PMon

In vivo imaging of hepatocellular carcinoma using a glypican-3-binding peptide based probe

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Hepatocellular carcinoma (HCC) has been the third most common cause of cancer-related death worldwide. Glypican-3 (GPC3) is a heparin sulfate proteoglycan linked to the cell membrane by a glycosyl-phosphatidylinositol anchor (GPI) and is expressed by 75% of all hepatocellular carcinomas but undetectable in healthy liver tissue or liver with focal lesions. What's more, GPC3 has been gradually applied in clinical applications as a specific indicator for the early detection and prognosis of HCC. As GPC3 can also regulate many pathways in HCC pathogenesis including Wnt, Hh and Yap signaling, it has been shown that GPC3 knockdown can inhibit HCC growth, reinforcing the important roles of GPC3 in HCC development. For HCC early detection, we designed a peptide targeting GPC3 that allows to establish a fluorescent dyes-labeled probe. Firstly, according to the structure of the GPC3 antibody GC33 and the positive peptide reported in the literature, we generated a peptide consisting of twelve amino acids named 12P that may bind to GPC3 with tight binding ability and specificity. In vitro testing, we utilized FCM and laser confocal microscopy to verify its specificity of targeting to the high expression cells of GPC3. What's more, we linked 12P with a near infrared dye to verify its in vivo targeting ability. All results indicated that 12P possessed potent binding capacity which could be used as a targeting module in GPC3 detection probe.

10079-28, Session PMon

Direct detection of microRNAs using isothermal amplification and molecular beacon with excellent sensitivity and specificity

Wancun Zhang, Qi Zhang, China Pharmaceutical Univ. (China); Zhiyu Qian, Nanjing University of Aeronautics and Astronautics (China); Yueqing Gu, China Pharmaceutical Univ. (China)

MicroRNAs (miRNAs) play important roles in a wide range of biological processes, including proliferation, development, metabolism, immunological response, tumorigenesis, and viral infection. The detection of miRNAs is imperative for gaining a better understanding of the functions of these biomolecules and has great potential for the early diagnosis of human disease as well as the discovery of new drugs through the use of miRNAs as targets. In this article, we developed a highly sensitive, and specific miRNA assay based on the two-stage exponential amplification reaction (EXPAR) and molecular beacon. The two-stage EXPAR involves two templates and two-stage amplification reactions under isothermal conditions. The first template enables the amplification of miRNA, and the second template enables the conversion of miRNA to the reporter oligonucleotide. Importantly, different miRNAs can be converted to the same reporter oligonucleotides seperately, which can hybridize with the same set of molecular beacon to generate fluorescent signals. This assay is highly sensitive and specific with a detection limit of 0.1 fM and can even discriminate single-nucleotide differences. Moreover, in combination with the specific templates, this method can be applied for multiplex miRNA assay by simply using the same molecular beacon. This method has potential to become a promising miRNA quantification method in biomedical research and clinical diagnosis.

10079-29, Session PMon

Features of software packages for multicolor flow cytometry: Introducing FULLBRIGHT, a versatile simulations workbench

Masahiko Taniguchi, Hai Du, Jonathan S. Lindsey, North Carolina State Univ. (United States)

Multicolor flow cytometry is a modern powerful analytical tool in widespread use for clinical assays. Yet as the number of fluorophores increases, the spectral overlap between fluorophores requires compensation of the emission spillover, complicating analyses and experimental designs.

A number of commercially available software packages have been developed. The limitations of such software often stem from the commercial interests of the flow cytometry instrument/dye vendor: (i) the fluorophores to be employed are limited to those pre-loaded in the software, (ii) excitation wavelengths are fixed to the specified laser lines of the given instrument, (iii) choice of channels (defined by bandpass filters) are limited to a given manufacturer's specifications, and (iv) the wavelength-dependent sensitivity of detectors (e.g., photomultiplier tubes) are rarely taken into consideration.

To overcome such limitations, we have developed a versatile software package, named "FULLBRIGHT", which works seamlessly as a module in the established PhotochemCAD program and databases. FULLBRIGHT is a researcher's workbench for calculating the brightness of fluorophores in a given wavelength range defined by bandpass filter channels. FULLBRIGHT contains the following databases: (i) absorption spectra of fluorophores with molar absorption coefficients, (ii) fluorescence spectra of fluorophores with quantum yields, (iii) spectra of bandpass filters and dichroic mirrors, and (iv) sensitivity curves of photomultiplier tubes. FULLBRIGHT is a versatile and flexible program - any excitation wavelengths can be applied, and the user can upload additional spectra as desired. FULLBRIGHT will be freely downloadable in due course at www.photochemcad.com.

10079-30, Session PMon

A novel sensitive fluorescent probe for H2O2 detection and its application in bio-imaging

Peng Wang, Jinxin Huang, Yueqing Gu, China Pharmaceutical Univ. (China)

To date, reactive oxygen species (ROS) have received considerable attention. Among different ROS, the role ofH2O2 as a second messenger, in regulating fundamental biological processes, has been identified not long ago and is increasingly supported by new data. Fluorescent H2O2 probes, designed to detect this oxygen metabolite with high selectivity, are powerful tools for real-time, noninvasive monitoring of H2O2 chemistry

in biological specimens. Herein, a turn-on fluorescent probe based on (E)-1-(naphthalen-2-yl)-3-phenylprop-2-en-1-one was synthesized for the sensitive detection of hydrogen peroxide. After the addition of hydrogen peroxide, an approximately 20-fold enhancement in fluorescence intensity at 620 nm was observed. There was a good linearity between relative fluorescent intensity at 620 nm and the concentration of H2O2 ranging from 0–100 ?M. The detection limit of this probe is 79 nM. The selectivity experiment indicated that this probe has reasonable activity and selectivity to identify H2O2 in a complex biological environment. MTT assay showed that cell viability was over 85% even though 50 ?M DCM-B2 was added for 24 h, indicating that the fluorescence probe had low cytotoxicity. Hence, it could offer good performance in terms of sensitivity, selectivity, and low cytotoxicity. Moreover, the potential of the probe as biosensor for hydrogen peroxide was demonstrated by imaging of hydrogen peroxide in living cells and tissues. This novel probe may present a promising tool to detect H2O2 during physiological and pathological processes.

10079-31, Session PMon

Novel magnetic graphene quantum dot as dual modality fluorescence/MMOCT contrast agent for tracking epithelial cells

Wei Li, Stephen J. Matcher, The Univ. of Sheffield (United Kingdom)

A novel nanoparticle, magnetic graphene quantum dot (MGQD) with fluorescence and superparamagnetic properties was synthesized for cell tracking using magnetomotive optical coherence tomography (MMOCT) and confocal fluorescence microscopy (CFM).

The MGQDs were synthesized by coating iron oxide on graphene oxide sheets, followed by the hydrothermal reaction in which the graphene oxide-iron oxide sheets were simultaneously reduced and cut in an autoclave at 200? for 10h forming quantum dots. The MGQD is a layered material several nanometres high and several microns in diameter. The magnetic hysteresis curves of MGQDs were measured by superconducting quantum interference magnetometer, which indicated that MGQDs had superparamagnetism and 41.7emu/g of saturation magnetization. MGQDs put on an agar surface for mimicking human tissue was imaged using an in-house MMOCT system. A strong magnetomotive signal of MGQD was detected in 0.08T of 80Hz alternating magnetic field. The magnetomotive signal was used to localise MGQD successfully in MMOCT image. The fluorescence of MGQD was measured by fluorescence spectrometer, which shows that the MGQD has excitation-depend fluorescence and emits visible fluorescence at peak of 420nm under the excitation of 370nm light. Therefore, the MGQD could be used as a contrast agent for tracking cells in MMOCT system and CFM. Use of MMOCT in vivo provides anatomical information in clinical applications with micron scale resolution and long imaging depth. MGQD labelled cells imaged by CFM can obtain intracellular details due to higher resolution, while CFM is unsuitable for imaging anatomical structure because of the limited view of field. The combination of the MMOCT and CFM images through the MGQD could give more comprehensive diagnosis.

10079-32, Session PMon

Quantification of non-specific binding by surface-enhanced Raman scattering detection of magnetically enriched and nanoparticle targeted cells

Wei Shi, Vincent Bouvet, Robert J. Paproski, Elizaveta Kharlova, Cody Bergman, Frank Wuest, Roger J. Zemp, Univ. of Alberta (Canada)

Detection of circulating tumor cells has tremendous diagnostic and prognostic significance but is difficult owing to low numbers of circulating tumor cells in large volumes of blood. Moreover, specificity of detection is non-trivial. We recently proposed a method for in vivo detection of circulating tumor cells using targeted magnetic and surface-enhanced Raman-scattering (SERS) nanoparticles. When circulating tumor cells are targeted with magnetic nanoparticles they can be magnetically trapped with a cone magnet. The trapped cells can be detected by detecting unique spectral signatures of the co-targeted SERS nanoparticles on these cells. To overcome challenges of targeting specificity, we introduce a multiplexed approach, where one flavor of SERS nanoparticle is targeted and another flavor is untartgeted. Ratiometric measurements permit assessment of specific versus non-specific binding. This is demonstrated using folate-targeting of HeLa cells with ZR-75-1 cells as a negative control. A non-specific binding ratio of 1:12(ZR-75-1 to HeLa) was measured. Further experiments were performed by using prostate cancer cell line LNCaP and PC3 with Prostate-Specific Membrane Antigen (PSMA)-targeted magnetic nanoparticles & SERS nanoparticles. The high specificity and magnetic enrichment of our method may prove important for cancer research, with in vivo application potential.

10079-33, Session PMon

Energy migration confinement in upconversion nanoparticles towards optimized photodynamic therapy effect

Yihua Zhao, Xiao Peng, Guangsheng Wang, Shuyi Yuan, Wei Yan, Jun Song, Junle Qu, Shenzhen Univ. (China)

Lanthanide doped upconversion nanoparticles (UCNPs) have received much attention due to their potential application in optical devices, sensing and therapeutics. Compared with conventional organic dyes and quantum dots, the UCNPs possess following advantages: near-infrared (NIR) excitation, low cytotoxicity, high chemical stability and low photo-bleaching. However, the commonly studied lanthnide activators such as Er3+ and Tm3+ contain abundant metastable excited states, and the dominant emission usually does not lie within the “tissue optical window” (spanning approximately from 650 to 1200 nm). Here, we present a core-shell-shell approach to tune the color emission of typical NaYF4:Yb/Er system. By embeding NaYF4:Yb/Er between NaYF4 layers, the energy migration induced energy loss to the crystal lattice in NaYF4:Yb/Er can be effectively suppressed. Moreover, the cross relaxation effect can be adopted to induce red color emission, without “concentration quenching effect”. The resulting UCNPs show low cytotoxicity, according to HeLa cell-based MTT assay. Furthermore, we have proven this optimized core-shell-shell structure can achieve efficient Förster resonance energy transfer process (FRET) for photodynamic therapy. In conclusion, we developed a novel core-shell-shell approach to boost both the red color emission and the luminescence efficiency of typical NaYF4:Yb/Er system, where a high PDT efficacy can be achieved.

10079-34, Session PMon

Nanoindentation of a new graphene/phospholipid composite: a numerical simulation

Olga E. Glukhova, Dmitriy S. Shmygin, Mikhail M. Slepchenkov, Saratov State Univ. (Russian Federation)

By means of an AMBER/AIREBO hybrid method we investigated indentation of layered graphene/phospholipids composite in which the individual phospholipid molecules arranged between the graphene layers. As a result of calculations it was established that such composite is characterized by negative enthalpy of reaction. An armchair carbon nanotube approaching with the speed of 10 m/s to the considered composite was used as an indenter. Previously, such method of numerical experiment with nanoindentation has been used to predict the behavior of high-density lipoprotein under mechanical load. All numerical experiments were carried out using original program KVAZAR (http://nanokvazar.ru/). During the simulation it was found that under the action of indenter

upper graphene layer in the composite starts to sag, exerting the pressure on the phospholipids which are located under it. Under the influence of pressure phospholipids begins to move on graphene trying to get away from the indenter. Therefore, by placing the phospholipids under improvised press it is possible to achieve their selective localization on graphene platform. The results of the calculations of the total energy of the studied molecular system showed that the value of energy begins to increase as the tube penetration deep inside the composite, indicating the loss of the structure stability. It was found that the strength of the layered graphene/phospholipids composite will increase with the increase in the number of graphene layers

10079-35, Session PMon

A new hybrid model to simulate interaction between DNA and carbon nanostructure

Olga E. Glukhova, George V. Savostyanov, Mikhail M. Slepchenkov, Artyom A. Zyktin, Saratov State Univ. (Russian Federation)

Currently, one of the key interdisciplinary scientific problems is to expand the knowledge in the field of prediction of the initial moment of disease at the atomic and cellular level based on the predictive molecular modeling. The reliable physical and mathematical tools capable to solve diverse problems in the development of new bioelectronic devices and predict the properties of new materials are needed. A vivid example is the problem of creation of highly selective biosensors. In particular, a new round of development in molecular electronics is associated with a DNA-based electronics. In this work a new hybrid theoretical model allowing us to investigate the interaction between the components of the DNA + carbon nanostructure molecular complex on the atomic and molecular levels is developed. Within the developed model we proposed to describe the carbon nanostructures by means of the methods and approaches of atomistic modeling, and to describe the DNA molecule using the methods and approaches of coarse-grained modeling. A coarse-grained structure of DNA is built based on 3-Site-Per-Nucleotide model. The proposed hybrid model has been implemented in the original software complex for molecular modeling KVAZAR using modern IT-solutions. The novelty of the model is concluded to a finding the weight coefficients for the interaction of large particles, simulating DNA, and conventional particle, simulating carbon nanostructure, and also for the intermolecular interactions. On the basis of established regularities for interaction between DNA and carbon nanostructures we will develop the model of the sensor device.

10079-36, Session PMon

Phospholipid dynamics in graphene of different topologies: predictive modeling

Olga E. Glukhova, Mikhail M. Slepchenkov, Saratov State Univ. (Russian Federation)

At present time graphene bioelectronics is actively developing scientific direction in the field of nanosystem industry. One of the most urgent tasks of a graphene bioelectronics is to develop optimal method for selective localization of phospholipid molecules on graphene in order to carry out an effective assembly of supramolecular structures. The subject of our scientific interest is the dynamics of the phospholipid molecules into a corrugated graphene sheet. According to our assumption by changing the topology of graphene properly it is possible to find the ways for management of the selective localization of phospholipid molecules to form the desired configuration of these structures. We considered DPPC (dipalmitoylphosphatidylcholine) phospholipids, which are the part of cell membranes and lipoproteins. We investigated the behavior of the phospholipids on the graphene sheet consisting of 1710 atoms with the size of 6.9 nm along the zigzag edge and 6.25 nm along the armchair edge. The numerical experiment was carried out using the original AMBER/AIREBO hybrid method with Lennard-Jones potential to describe the interaction between unbound atoms of different structures. The temperature was maintained at 300 K during the numerical experiment. All numerical experiments were performed using KVAZAR software system. We considered several cases of corrugated graphene with different width and height of the corrugation. Special attention in our work was paid to the orientation of the phospholipids in the plane of graphene sheet.

10079-21, Session 7

Copolymerized and bonded fluorescent silica nanoparticles as labels and pseudostationary phase in bioanalytical applications *(Invited Paper)*

Gabor Patonay, Maged M. Henary, Walid Abdelwahab, Gala Chapman, Georgia State Univ. (United States)

Silica nanoparticles have been increasingly used in developing bioanalytical, biomedical and in many other applications. Silica nanoparticles can easily be synthesized and with the advent of wide availability of modified TEOS reactive analogues only the researcher imagination is the limit of preparing silica nanoparticles that contain different molecules that are either copolymerized inside of the silica nanoparticle or chemically attached (bonded) to the silica nanoparticle surface. Relatively non-porous silica nanoparticles can contain copolymerized dyes for the creation of bright fluorescence labels while the surface of these silica nanoparticles can be bonded with reactive moieties that are suitable for covalently labeling the molecule of interest. Also the surface bonded moieties can serve other purposes, e.g., molecular recognition either on a non-fluorescent or fluorescent silica nanoparticle. As far as the fluorescent nanoparticles development concerns near-infrared (NIR) absorbing carbocyanine dyes have been increasingly used as they can be useful for developing bioanalytical, biomedical methods and in many other applications. Carbocyanines are preferred as they are relatively easy to synthesize and can be designed to achieve particular spectroscopic properties. For example either copolymerized or surface bound dyes can contain appropriate functional moieties absorption and fluorescence properties change when it is complexed to metal ions, to detect pH changes, bind to biological molecules, etc. Fluorescence intensity of carbocyanines significantly increases by enclosing several dye molecules in a single silica nanoparticle due to shielding however self quenching may become a problem at high dye concentrations in confined spaces. Large Stokes' shift dyes can significantly decrease this problem. This can be achieved by substituting meso position halogens in the NIR fluorescent carbocyanines with a linker containing amino moiety which can also serve as linker to covalently attach the dye molecule during the nanoparticle synthesis. This presentation discusses facile synthesis and applications of silica nanoparticles containing copolymerized fluorophores and/or surface bound moieties. Applications include silica nanoparticles containing several dye molecules as bright labels in immunochemical uses, cell imaging and forensic applications for latent blood detection. This latter application was developed using leuco fluorescein copolymerized silica nanoparticles. This synthesis proved that copolymerized dyes can be further modified after the dye containing silica nanoparticle was formed. Surface bound moiety examples will be given for capillary electrochromatography using amino acid-bonded silica nanoparticles as pseudostationary phases as chiral selectors.

10079-22, Session 7

Optical and magnetic characterization of theranostic magnetite particles *(Invited Paper)*

Dana Cialla-May, Leibniz-Institut für Photonische Technologien e.V. (Germany); Sophie Patze, Leibniz-Institut

für Photonische Technologien e.V. (Germany); Robert Mueller, Karina Weber, Jürgen Popp, Leibniz-Institut für Photonische Technologien e.V. (Germany)

Magnetic nanoparticles (MNPs) have a major role as contrast agent in diagnostic imaging and therapeutic monitoring. In order to research on MNP exposition, degradation and elimination of those nano composites as well as the consequences of the MNP exposition in relation with social economic relevant diseases (cancer, infectious diseases), the comprehensive characterization of magnetic and structural properties is of high importance. Within this contribution, the magnetic characterization of theranostic relevant MNPs is introduced. Applying a vibrating sample magnetometer (VSM), it is found, that the nanocomposites show superparamagnetic behavior and the recorded data confirm iron oxide cores (magnetite/maghemite). Employing Raman spectroscopy, the typical fingerprint information of magnetite is detected. By increasing the laser power, the transition to maghemite and hematite due to the oxidation of the magnetic core is illustrated. Moreover, IR spectroscopy is applied to characterize the coating material e.g. starch or other biocompatible polymers. To determine the stability of MNPs as well as the MNP's elimination under physiological conditions, different buffer systems were tested i.e. simulated body fluid (SBF) and artificial lysosomal fluid (ALF). The investigated MNPs are stable in SBF; thus, the stability in blood after injection of the contrast agent is guaranteed. Finally, the storage in ALF leads to a complete decomposition of the MNPs, which reflects the conditions in lysosomes and guarantee for a fast MNP elimination.

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10079-38, Session 7

Future of fluorescent nanodiamonds

Philip R. Hemmer, Texas A&M Univ. (United States)

Recently there has been significant interest in using fluorescent nanodiamonds (FND) for bio-imaging applications. There are several reasons for this: 1) FND has very low toxicity compared to other fluorescent nanoparticles and dyes, 2) FNDs are generally non-bleaching and non-blinking, 3) FND can be made smaller than other nanoparticles while still retaining its bulk properties (< 2 nm demonstrated). However to realize the full potential of FND, a new approaches to nanodiamond fabrication are required. I will review current diamond fabrication techniques to illustrate the limitations, and then I will discuss new approaches for overcoming these. In particular I will discuss new deterministic approaches to FND fabrication that promises to allow precise engineering of FNDs.

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10080-1, Session 1

Surface-enhanced near-infrared absorption on nanoporous gold nanoparticle array chip

Wei-Chuan Shih, Fusheng Zhao, Univ. of Houston (United States)

Near-infrared (NIR) absorption spectroscopy in the 1-2.5 ?m wavelength range can provide chemical information based on the overtones and combination bands of fundamental vibrational modes in the infrared (IR) wavelength range. NIR absorption features are significantly broader and weaker due to the fact that the underlying processes are quantum mechanically forbidden. However, substantially lower water absorption allows NIR spectroscopy to be performed on samples with high water content, e.g., biological specimen and other in situ measurements, which otherwise restricts the use of IR light. However, small NIR absorption cross-section results in less sensitivity compared to measuring the IR fundamentals. In addition, NIR measurements are more challenging compared to in other spectral regions because of the lack of high-sensitivity detectors. To overcome these barriers, we propose the use of plasmonic nanostructures.

Nanoporous gold nanoparticle (NPG-NP) array chip showcases tunable pore and ligament sizes ranging from nanometers to microns. The nanoporous structure and sub-wavelength nanoparticle shape contribute to its unique LSPR properties. NPG-NP features large specific surface area and high-density plasmonic field enhancement known as “hot-spots”. Hence, NPG-NP has found many applications in nanoplasmonic sensor development. In our recent studies, we have shown that NPG-NP array chip can be utilized for high-sensitivity detection by various enhanced spectroscopic modalities, as photothermal agents, and for disease biomarker detection.

In this paper, we show the first experimental demonstration of effective and robust surface-enhanced near-infrared absorption (SENIRA) on NPG-NP array chip.

10080-2, Session 1

Guided-mode-resonance coupled localized surface plasmons for dually resonance enhanced Raman scattering sensing

Zheng Wang, Chao Liu, The Univ. of Texas at Austin (United States); Erwen Li, Oregon State Univ. (United States); Swapnajit Chakravarty, Xiaochuan Xu, Omega Optics, Inc. (United States); Alan X. Wang, Oregon State Univ. (United States); D. L. Fan, The Univ. of Texas at Austin (United States); Ray T. Chen, The Univ. of Texas at Austin (United States) and Omega Optics, Inc. (United States)

Raman scattering spectroscopy is a unique tool to probe vibrational, rotational, and other low-frequency modes of a molecular system and therefore could be utilized to identify chemistry and quantity of molecules. However, the ultralow efficient Raman scattering, which is only 1/109 ~ 1/1014 of the excitation light due to the small Raman scattering cross-sections of molecules, have greatly hindered its development in practical sensing applications. The discovery of surface enhanced Raman scattering (SERS) in 1970s and the significant progresses in nanofabrication technique, provide a promising solution to overcome the intrinsic issues of Raman spectroscopy. It is found that In the vicinity of nanoparticles and their junctions, the Raman signals of molecules can be significantly improved by an enhancement factor as high as 1010, due to the ultrahigh electric field generated by the localized surface plasmons resonance (LSPR), where the intensity of Raman scattering is proportional to the |E|4. Recently double-resonance plasmonic substrates, which could combine LSPR from “hot spots” and surface plasmons polaritons (SPPs) from the underneath substrate, have been demonstrated to be a promising approach for SERS substrates. However, the large distance among different “hot spots” (350 nm ~ 780 nm) makes the total SERS effect low. In this work, we propose and demonstrate a new approach combining LSPR from nanocapsules with densely assembled silver nanoparticles (NC-AgNPs) and guide-mode-resonance (GMR) from dielectric 2D photonic crystal slab (PCS) for SERS substrates with robustly high performance.

10080-3, Session 1

Contact point SERS based on plasmonic crystal shells

Xiangwei Zhao, Southeast Univ. (China)

SERS attracts more and more attentions in biomedical fields. However, how to obtain high intensive and reproducible SERS signals is still challenging because lots of factors involved, one of which is the co-localization of the analytes and the hot spots. In this paper, we present a strategy of fabricating plasmonic crystal shell based on photonic crystal beads with electroless plating for high performance SERS application. When the shell containing solution dries on a glass slide, the analyte will precipitate at the contact point of the shell and substrate, which make it co-localize with the hot spots of the plasmonic crystal for high sensitive and reproducible SERS detection. By confining the seeding zone in electroless plating, closed and open plasmonic shells were fabricated. It is shown that hot spots of open shells have ca. four times of electric filed intensity than that of closed ones in not only simulations but also SERS experiments. In order to demonstrate the performance of the plasmonic crystal shells, Raman probe R6G was used and LOD of 10-12 M was obtained with high reproducibility. The plasmonic crystal shell provides a new substrate for SERS detection in biomedical fields and holds a great promise for practical applications.

10080-4, Session 1

Intensified surface enhanced Raman signal of a graphene monolayer on a plasmonic substrate through the use of fluidic dielectrics

Amirreza Mahigir, Manas Ranjan Gartia, Louisiana State Univ. (United States); Te-Wei Chang, Intel Corp. (United States); Gang Logan Liu, Univ. of Illinois at Urbana-Champaign (United States); Georgios Veronis, Louisiana State Univ. (United States)

It has been shown that surface enhanced Raman spectroscopy (SERS) has many promising applications in ultrasensitive detection of Raman signal of substances. However, optimizing the enhancement in SERS signal for different applications typically requires several levels of fabrication of active plasmonic SERS substrates. In this paper, we report the enhancement of SERS signal of a single layer of graphene located on a plasmonic nano-Lycurgus cup array after placing water droplets on it. The experimental data shows that addition of water droplets can enhance the SERS signal of the single layer of graphene about 10 times without requiring any modifications to the nano-Lycurgus cup array. Using full-wave electromagnetic simulations, we show that addition of water droplets enhances the local electric field at the graphene layer, resulting in stronger light-graphene interaction at the excitation pump laser wavelength. We also show that the addition of water droplets on the graphene layer enables us to modify the band diagram of the structure, in order to enhance the local density

of optical states at the Raman emission wavelengths of the graphene layer. Numerical calculations of both the excitation field enhancement at the location of the graphene layer, and the emission enhancement due to enhanced local density of optical states, support the experimental results. Our results demonstrate an approach to boost the SERS signal of a target material by controlling the band diagram of the active nanostructured SERS substrate through the use of fluidic dielectrics. These results could find potential applications in biomedical and environmental technologies.

10080-5, Session 1

Sensitive and selective nanoplasmonic sensor by functionalized nanoporous gold nanoparticle array chip

Fusheng Zhao, Suyan Qiu, Jingting Li, Wei-Chuan Shih, Univ. of Houston (United States)

Localized surface plasmon resonance (LSPR) arises from the interaction of light with noble metal nanoparticles, which induces a collective oscillation in the free electrons. The size and shape of the metallic nanostructure significantly impact LSPR frequency and strength. Nanoplasmonic sensor has become a recent research focus due to its significant signal enhancement and robust signal transduction measured by extinction spectroscopy, fluorescence, Raman scattering, and absorption spectroscopy. However, since the native gold surface does not have the capability to selectively bind target biomolecules, high molecular specificity has been a challenge.

Nanoporous gold nanoparticle (NPG-NP) array chip showcases tunable pore and ligament sizes ranging from nanometers to microns. The nanoporous structure and sub-wavelength nanoparticle shape contribute to its unique LSPR properties. NPG-NP features large specific surface area and high-density plasmonic field enhancement known as “hot-spots”. Hence, NPG-NP has found many applications in nanoplasmonic sensor development. In our recent studies, we have shown that NPG-NP array chip can be utilized for high-sensitivity detection by various enhanced spectroscopic modalities, as photothermal agents, and for disease biomarker detection.

To improve sensing specificity, gold surface can be decorated with moieties to promote selective binding of target molecules. In this paper, we discuss strategies to enhance molecular specificity by functionalizing NPG-NP with unique bio-recognition elements towards both high sensitivity and specificity. A few examples will be given using existing and novel bio-recognition elements.

10080-6, Session 1

20 years of quantum dots for biophotonics and nanomedicine *(Invited Paper)*

Ken-Tye Yong, Nanyang Technological Univ. (Singapore)

During the last 20 years, QDs have been applied in healthcare applications such as cancer imaging, lymph node mapping and brain diseases therapy. These nanocrystals can be engineered to serve as a platform for challenges in highly sensitive optical diagnostic tools, biosensors, and guided imaging and therapy. QDs are luminescent semiconductor particles in the nanometer sizes and they possess many unique optical properties, which have significant advantages over traditional organic dyes as luminescence marker for biological applications. For example, QDs that fluoresce in different colors can be simultaneously excited with a single light source, with minimal spectral overlap. In addition, QDs can be made to emit in a range of wavelengths by changing their size, shape, and composition. This flexibility in optical tuning allows QDs to emit from visible to near-infrared (NIR) region, an essential characteristic for tailoring specific needs in biophotonic applications. In this talk, we will highlight the use of QDs with different sizes, compositions, and shapes for biophotonic applications (e.g. guided bioimaging, multimodal imaging, sensing, in vivo surgery, gene delivery, etc). Also, we will discuss important factors to be considered when designing bioconjugated QDs for in vitro and in vivo applications and the future trend of using QDs in the biophotonic field. Certainly, the in vitro and in vivo nanotoxicity of QDs will be one of the main challenges to be overcome in the near future if we would like/want to pursue in vivo biophotonic technologies with QDs. The toxicity assessment of QDs in cell culture and animal models will be presented. This talk is intended to promote the awareness of past and present developments of QD in biomedical fields, the potential toxicity of QDs, and the approaches to engineer new types of QDs, whereby encouraging researchers to think about exciting and promising biophotonic applications with QDs in the near future.

10080-7, Session 1

Plasmonics-based SERS nanobiosensor for gastrointestinal cancer diagnostics via microRNA biomarker detection

Bridget M. Crawford, Hsin-Neng Wang, Andrew M. Fales, R. Von Furstenberg, K. Garman, Tuan Vo-Dinh, Duke Univ. (United States)

No Abstract Available

10080-8, Session 1

SERS-based application in food analytics

Dana Cialla-May, Andreea Radu, Leibniz-Institut für Photonische Technologien e.V. (Germany); Martin Jahn, Leibniz-Institut für Photonische Technologien e.V. (Germany); Karina Weber, Jürgen Popp, Leibniz-Institut für Photonische Technologien e.V. (Germany)

To establish detection schemes in life science applications, specific and sensitive methods allowing for fast detection times are required. Due to the interaction of molecules with strong electromagnetic fields excited at metallic nanostructures, the molecular fingerprint specific Raman spectrum is increased by several orders of magnitude. This effect is described as surface-enhanced Raman spectroscopy (SERS) and became a very powerful analytical tool in many fields of application. Within this presentation, we will introduce innovative bottom-up strategies to prepare SERS-active nanostructures coated with a lipophilic sensor layer. To do so, the food colorant Sudan III, an indirect carcinogen substance found in chili powder, palm oil or spice mixtures, is detected quantitatively in the background of the competitor riboflavin as well as paprika powder extracts. The SERS-based detection of azorubine (E122) in commercial available beverages with different complexity (e.g. sugar content, alcohol concentration) illustrates the strong potential of SERS as a qualitative as well as semiquantitative prescan method in food analytics. Here, a good agreement between the estimated concentration employing SERS as well as the gold standard technique HPLC, a highly laborious method, is found. Finally, SERS is applied to detect vitamin B2 and B12 in cereals as well as the estimate the ratio of lycopene and ?-carotene in tomatoes.

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10080-29, Session 1

Plasmonic SERS nanochips and nanoprobes for medical diagnostics and bioenergy applications

Hoan Thanh Ngo, Hsin-Neng Wang, Bridget M. Crawford, Andrew M. Fales, Tuan Vo-Dinh, Duke Univ. (United States)

No Abstract Available

10080-9, Session 2

3D plasmonic crystal metamaterials for ultra-sensitive biosensing

Artem Danilov, Andrei V. Kabashin, Aix-Marseille Univ. (France); Maria Manousidaki, Maria Farsari, Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas (Greece)

We show that the employment of 3D nanoarchitectures of plasmon excitation can break the diffraction-related limitation of 2D periodic structures and thus obtain a much improved spectral sensitivity (2600 nm/RIU), as well as a prominent phase response (3*10^4 Deg. of phase per RIU). The sensitivity enhancement was demonstrated using Woodpile-based photonic crystal metamaterials, but we believe that similar effect can be obtained with alternative 3D architectures (nanorod etc) constituting effective metal/dielectric medium. One of the key advantages of the proposed metamaterial-based transducer geometry consists in the large area for biomolecule immobilization offered by the 3D matrix. This enables the implementation of new sensing geometries and strategies, not feasible with film-based SPR or 2D LPR. Indeed, by functionalizing the woodpile blocks and immobilizing a receptor on their surfaces, one can follow the binding of a selective analyte with the receptor inside the woodpile matrix. The considerably increased surface area given by the metamaterial topography significantly increases the amount of biomaterial that can be incorporated into the matrix within the available probe depth, maximizing the “biological” sensitivity of the system. Furthermore, the distance between the woodpile blocks can be selected to match the size of biological species of interest, giving access to a further size selectivity option that is important for many tasks in immunoassays and virus and protein detection. In general, relatively large openings in the woodpile array could contribute to a good transport of biological species, which is not always the case when porous nanomaterials are used as bioimmobilization templates. Finally, a strong field enhancement at some points of the metamaterial matrix makes possible the involvement of an additional Surface Enhanced Raman Scattering (SERS) channel, which can be used in parallel with the main optical transduction channel.

10080-10, Session 2

Nitrogen doped graphene quantum dots effectively preserve the surface enhancement performance of silver nanoparticles

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In this work, we report a novel substrate for surface enhanced Raman spectroscopy (SERS) composed of silver nanoparticles protected by small nitrogen-doped Graphene Quantum Dots, i.e. Ag NPs-N-GQDs, synthesized under mild experimental conditions, which can preserve the SERS performance in normal indoor environment for up to 30 days. The field emission scanning electronic microscope (FESEM) images confirm that the N-GQDs play a significant role in the control of metallic nanoparticles morphology. The X-ray photoelectron spectroscopy (XPS) result clearly indicates the N-GQDs was successfully immobilized on the surface of silver nanoparticles (Ag NPs). Ag NPs-N-GQDs demonstrated Raman enhancement stronger than pure Ag NPs likely due to an increase in the number of the “hotspots” formed by coupled nanostructures. N-GQD protected Ag NPs were evaluated in SERS measurements of R6G when they were made fresh and have been stored in normal indoors condition for up to 30 days. Then Ag NPs-N-GQDs were used as a SERS substrate for glucose detection. The linearity range of glucose was found to be ranged from 1 ?M to 1 M with a detection limit of 0.1 ?M in glucose solutions. It was also applied successfully for glucose detection in rat blood samples. The present study demonstrates that the novel Ag NPs?N-GQDs nanostructure has great potential to be used as a cost effective sustained SERS substrate, which can be extremely useful in the wide adoption of SERS based sensors.

10080-11, Session 2

Localized surface plasmon enhanced cellular imaging using random metallic structures

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We have studied fluorescence cellular imaging with randomly distributed localized near-field induced by silver nanoislands. For the fabrication of nanoislands, a 10-nm silver thin film evaporated on a BK7 glass substrate with an adhesion layer of 2-nm thick chromium. Micrometer sized silver square pattern was defined using e-beam lithography and then the film was annealed at ~ 200°C. Raw images were restored using electric field distribution produced on the surface of random nanoislands. Nanoislands were modeled from SEM images. 488-nm p-polarized light source was set to be incident at 60°. Simulation results show that localized electric fields were created among nanoislands and that their average size was found to be ~135 nm. The feasibility was tested using conventional total internal reflection fluorescence microscopy while the angle of incidence was adjusted to maximize field enhancement. Mouse microphage cells were cultured on nanoislands, and actin filaments were selectively stained with FITC-conjugated phalloidin. Acquired images were deconvolved based on linear imaging theory, in which molecular distribution was sampled by randomly distributed localized near-field and blurred by point spread function of far-field optics. The optimum fluorophore distribution was probabilistically estimated by repetitively matching a raw image. The deconvolved images are estimated to have a resolution in the range of 100-150 nm largely determined by the size of localized near-fields. We also discuss and compare the results with images acquired with periodic nanoaperture arrays in various optical configurations to excite localized plasmonic fields and to produce super-resolved molecular images.

10080-12, Session 2

Detection of small molecules with surface plasmon resonance by synergistic plasmonic effects of nanostructured surfaces and graphene

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Surface plasmon resonance depends on the dielectric medium at the vicinity and makes it a quasi-universal detector. Therefore, and due to the label-free nature, SPR is a widely used sensing tool for real?time monitoring molecular interactions of various analytes. However, detection of highly diluted concentrations and small molecules (< 400 Da) is still challenging. Nanostructured gold films provide plasmonic hotspots with improved surface sensitivity and 2D carbon nanomaterials enable binding in close proximity to the surface. Both effects combined are promising in the development of SPR sensors for the efficient determination of small molecules.

The SPR characteristics of the SPR substrate, fabricated by nanosphere lithography and subsequent spin coating of reduced graphene oxide (rGO), were analyzed using the Kretschmann configuration at a fixed wavelength. Graphene is known for efficient binding of molecules with delocalized aromatic ϖ-systems. Additionally, the electromagnetic field is locally enhanced and modulated by the interaction of graphene photonics with the plasmonics of metal nanostructures.

In comparison to sensor substrates consisting of a continuous metal film the surface sensitivity is enhanced for a nanohole arrays, depending on the dimensions and density of these nanostructures. The enhanced sensitivity was proven for the detection of small molecules like plasticizers and purine-based biomarkers. The feasibility of the sensor was demonstrated for the detection of adenine down to a concentration of 2 μM, which is in the physiological relevant range.

10080-20, Session 2

The issue of limit of detection in surface plasmon resonance biosensors

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Surface plasmon resonance (SPR) biosensors have been widely reported as a label-free detector for real-time monitoring binding between biomolecules. Limit of detection (LOD), a term interchangeable with sensitivity, has always been an issue to assess the performance of sensors. Until now, there is no unified standard for SPR biosensors. Both ng/ml and refractive index unit (RIU) in the order of 10-6 RIU have been quoted extensively in the literature. But they actually have quite different implications. And there is also no established practice to experimentally estimate LOD. This presentation aims to provide a review on the current situation. Various scientific aspects of LOD in SPR biosensors are also highlighted.

10080-13, Session 3

Optical manipulation and catalytic activity enhanced by Surface plasmon effect *(Invited Paper)*

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For optical manipulation, a nano-optical conveyor belt consisting of an array of gold plasmonic non-concentric nano-rings (PNNRs) is demonstrated for the realization of trapping and unidirectional transportation of nanoparticles by polarization rotation of excitation beam. These hot spots of an asymmetric plasmonic nanostructure are polarization dependent, therefore, one can use the incident polarization state to manipulate the trapped targets. Trapped particles could be transferred between adjacent PNNRs in a given direction just by rotating the polarization of incident beam due to unbalanced potential. The angular dependent distribution of electric field around PNNR has been solved using the three-dimensional finite-difference time-domain (FDTD) technique. For optical enhanced catalytic activity, the spectral properties of dimers of Au nanorod-Au nanorod nanostructures under the excitation of 532nm photons have been investigated. With a super-resolution catalytic mapping technique, we identified the existence of "hot spot" in terms of catalytic reactivity at the gap region within the twined plasmonic nanostructure. Also, FDTD calculation has revealed an intrinsic correlation between hot electron transfer.

10080-14, Session 3

Ultra-sensing with slit-enhanced infrared spectroscopy

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Infrared spectroscopy enables the label-free detection of structure specific fingerprints of analytes. The sensitivity of corresponding methods can strongly be enhanced by attaching analytes on plasmonic active surfaces.

We introduce a slit array metamaterial perfect absorber (SAMPA) [1] consisting of a dielectric layer sandwiched between two Au layers of which the upper layer is perforated with a periodic array of slits. This structure combines the principle of Extraordinary Optical Transmission (more light is transmitted through a hole than is incident on its surface) with that of Perfect Absorption (reflectance and transmittance are virtually zero). Accordingly, within the slights the electric fields are strongly enhanced and light-matter interaction is correspondingly greatly amplified. Thus, already small concentrations of analytes down to a monolayer can be detected and identified by their spectral fingerprints with a standard mid-infrared spectrometer.

Closely related to the SAMPAs are plasmonic slit absorbers, which simply consist of slit arrays in thin gold layers deposited on a layer of Si3N4. [2] These slit arrays operate like unstructured gold layers if the incident light is polarized parallel to the long slit axes. In contrast, for light polarized perpendicular to the long slit axis, the plasmon is excited. By the introduction of a second slit, which is rotated relative to the first slit, both principal polarization states excite plasmon resonances which can be made to differ in wavelength. As a consequence, the operating wavelength range of this slit array can be tuned by adjusting the polarization state of the incoming light.

[1] Mayerhöfer, T.G., et al.. ACS Photonics, 2015. 2(11): p. 1567-1575.

[2] Knipper, R., et. al., in preparation.

10080-15, Session 3

Plasmonic distributed feedback cavity with a phase shift on single-mode optical fiber end facet for label-free biosensing

Zeyu Lei, Xin Zhou, Jie Yang, Xiaolong He, Tian Yang, Shanghai Jiao Tong Univ. (China)

Surface plasmon resonance (SPR) devices have been widely used in label-free biosensing applications due to their convenient surface wave configuration and the capability to optically detect biomolecule surface binding with a high stability and uniformity between different experiments. Meanwhile, integrating SPR nanostructures onto single-mode fiber (SMF)

end facets provides unique advantages such as flexible geometry, compact sizes and in vivo monitoring capability. To improve the performance of SMF end facet SPR devices which are usually limited by guided mode diffraction, following our previous work on plasmonic crystal cavities [1], in this work we demonstrate a plasmonic distributed feedback (DFB) cavity with a phase shift section. The DFB structure contains a periodic array of nanoslits in a gold film, which provides a surface plasmon polariton (SPP) bandgap from 865 to 877 nm on the water-gold interface. A phase shift section is embedded at the center of the DFB structure to introduce an SPR defect state within the SPP bandgap. The devices were fabricated onto the fiber end facets by a glue-and-strip transfer process [1]. To demonstrate real biosensing implementations, the reflection spectra of the SMF guided lightwaves were taken in real-time to detect refractive index change, adsorption of bovine serum albumin onto gold surface, and the association and dissociation between human immunoglobulin G (hIgG) and its antibody.

[1] X. He, H. Yi, J. Long, X. Zhou, J. Yang and T. Yang, "Plasmonic Crystal Cavity on Single-Mode Optical Fiber End Facet for Label-Free Biosensing," Applied Physics Letters 108, 231105 (2016)

10080-16, Session 3

Machine learning-assisted hyperspectral analysis of plasmonic contrast agent microbiodistribution with single-particle sensitivity and sub-cellular resolution

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Nanoparticles have been explored extensively as potential biomedical imaging and therapeutic agents. One critical aspect of in vivo nanoparticle use is the characterization of biodistribution profiles. Such studies improve our understanding of particle uptake, specificity, and clearance mechanisms. Currently, the most prevalent nanoparticle biodistribution methods provide either aspatial quantification of whole-organ particle accumulation or nanometer-resolution images of uptake in single cells. Few existing techniques are well-suited to study particle uptake on the micron to millimeter scales relevant to sub-tissue physiology. Here we demonstrate a new method called ABIDE (Automated Biodistribution Detection) that uses machine learning classification of hyperspectral dark-field images to study interactions between tissues and administered nanoparticles. This label-free, non-destructive method enables quantitative particle identification in histological sections and detailed observations of sub-organ accumulation patterns consistent with organ-specific clearance mechanisms, particle size, and the molecular specificity of the nanoparticle surface. Unlike studies with electron microscopy, ABIDE is readily applied for large fields of view. ABIDE achieves excellent detection sensitivity (99.4%) and specificity (99.7%) and can identify single nanoparticles. To demonstrate ABIDE's potential for novel nanoparticle uptake studies, we collected the first data on the sub-organ localization of large gold nanorods (LGNRs) in mice. We also observed differences in particle accumulation and localization patterns in tumors as a function of conjugated molecular targeting moieties. Thus, ABIDE affords new degrees of detail for the study of nanoparticle uptake at physiological scales. ABIDE may offer an auxiliary or alternative approach to study the biodistribution profiles of existing and novel nanoparticles.

10080-17, Session 3

Fiber optic based sensor for the detection of triacylglycerides using Ag/ZnO nanorods/lipase enzyme

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A surface plasmon resonance based fiber optic sensor for the detection of triacylglycerides (TG) has been fabricated by immobilizing lipase enzyme (Lip11) on Zinc Oxide (ZnO) nanorods grown over a silver (Ag) film. The sensor probe exhibits high sensitivity for 0–500 mg/dl triacylglyceride concentration, which covers both the physiological range and high levels (which indicate coronary heart disease and hyperlipoprotenimia). After coating a silver layer of 40 nm on the fiber surface by the thermal evaporation, the ZnO nanorods are grown over the silver layer by first preparing nanoparticles (NPs) of ZnO using the Pacholski method. The silver coated fiber is then dipped into the ZnO nanoparticles solution and the ZnO NPs work as a seed layer for the growth of ZnO nanorods on the fiber surface. Here, ZnO nanorods play a double role in the sensing operation. They act as a matrix with high isoelectric point (9.5) which makes it possible to directly immobilize lipase enzyme which has low isoelectric point (4.9) by electrostatic adsorption without any requirement of functionalization. Second the ZnO nanorods layer enhances the sensitivity of the sensor as it works as a high index layer over the metal layer. As the concentration of TG change in the vicinity of the sensor, the refractive index and thickness of bio-recognition enzyme layer change and as a consequence the resonance wavelength in the absorbance spectra changes. The sensor has a fast response, high selectivity and sensitivity, low cost, label free detection and ease of fabrication.

10080-18, Session 3

Effect of atmospheric pressure plasma on antimicrobial activity of cotton fabrics dyed with Zataria multiflora Bioss extract

Soudabeh Hajahmadi, Islamic Azad Univ. of Najafabad (Iran, Islamic Republic of)

Nowadays it is very important to finish almost all apparels with antimicrobial agents, many of the synthetic antimicrobial agents for textiles cause environmental deterioration and health problems for consumers, therefore bio materials which inhibit the growth of microorganism without causing health problem are becoming more attractive. Zataria multiflora Boiss. (ZM) is a popular, medicinal plant with a remarkable antibacterial and antioxidant activity, the antimicrobial activity of cotton fabrics treated with atmospheric pressure plasma (APP), dyed with ZM extract was evaluated against some gram negative and gram positive bacteria . Influence of plasma treatment on durability of antimicrobial activity of ZM on fabrics to light and washing also measured according to I.S.O. standard recommendations. The color strength of samples was analyzed using a Reflective Spectrophotometer.

The results show that using APP technology could play a beneficial role in the biomedical performance of cotton fabrics, higher inhibition rate amongst all bacteria was obtained more than 97% microbial reduction in all bacterial population. The results also show that, the color strength and fastness of samples have been improved after plasma treatment, a high antimicrobial activity about 92% in the case of using APP after exposure to light and washing was observed, up to 55% more than untreated samples.

Atmospheric pressure plasma can be used successfully for treating cotton fabrics dyed with ZM for use as bioactive textile.

10080-21, Session PMon

Preliminary study in plasmonic enhancements of nanostructured substrates for high-sensitive albumin detection

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Albumin is one of the important and abundant molecules in human. The albumin is not only key components of blood plasma, but also has clinical importance to employ one of the biomarkers for pre-clinical diagnosis. For this reason, high-performance bio-sensing and quantification of albumin from the specimen is significant issue in both biological research and clinical areas.

On the other hand, plasmonic light enhancement using field localization in metallic nanostructures has been investigated. Through previous theoretical and experimental studies, the plasmonic enhancement could be integrated to biological and biomedical labeled/label-free sensing applications such as surface plasmon resonance (SPR) biosensors and enhanced fluorescence sensors.

In this study, we have a preliminary study in plasmonic enhancements of metallic nanostructured substrates for the investigation of high-sensitive albumin detection. To be specific, we designed and fabricated two types of metallic nanostructured substrates (randomly deposited nanoscale islands and periodically distributed nanoscale bow-tie structures) for enhancing fluorescence signals from a fluorescent serum albumin indicator (Albumin 580). With preliminary study using various concentrations of bovine albumin serum (BS) with Albumin 580, efficient nanostructured substrates to monitor albumin with high-performance are determined. The result of this study can be practically used to investigate high-performance plasmonic fluorescence enhanced biological and biomedical sensors.

10080-22, Session PMon

LoC-SERS toward clinical application: quantification of antibiotics in human urine samples

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Fixed dose medical treatments derived from clinical studies carried out with healthy volunteers fail in the case of specific patients. Personalized medicine, where the treatment is tailored to the individual patient, should be, therefore, highly encouraged. The determination of the concentration of xenobiotics in biological matrix followed by the change of the prescribing procedure plays a major role.

Among the newly emerging analytical tools, lab-on-a-chip surface enhanced Raman spectroscopy (LoC-SERS) was proven to be suitable for the determination of low molecular weight substances with high specificity, sensitivity and sample throughput.

For this contribution, human urine samples collected from healthy volunteers and from patients having urinary tract infection were used as biological matrix to assess the potential and limitation of LoC-SERS to detected levofloxacin and nitroxoline.

Levofloxacin determination in the clinically relevant concentration range (1.38 mM ± 0.68 mM) is carried out by a bench-top and a portable Raman setup in six spiked urine samples. For nitroxoline, the successful detection at minimum inhibitory concentration of uropathogens (10-40 μM) in three spiked samples be presented. This is followed, by demonstrating the quantification power of LoC-SERS combined with the standard addition method applied to nitroxoline in simulated clinical samples.

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10080-23, Session PMon

Analysis of curvature effects on plasmon biosensing of molecular interactions

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Surface plasmon represents oscillations of electrons at the interface between metal and dielectric layers. Surface plasmon resonance (SPR) is influenced by the environment near the surface, which has been the basis for label-free biosensor structure for various applications of molecular detection. An important aspect of SPR biosensing is that its characteristics is affected by the geometrical structure. Yet most research focused largely on a structure using flat surface. Although flat structure is suitable for typical sensor applications, it may not be appropriate for wearable or in vivo applications. In this study, we analyzed the effects of surface curvature on flexible SPR biosensors. For convenience, curved surface was approximated using a segmented model in which each segment is treated as a flat surface with different incident angle and then optical characteristics of the overall model were calculated by rigorous coupled wave analysis in two different configurations of light incidence. We calculated curvature effects on SPR with curvature radius in a range of r > 255 um. The results suggest that regardless of the incident configurations, resonance curves tend to broaden with increased curvature due to larger momentum dispersion. Resonance shifts as a result of biosensing, such as DNA immobilization and hybridization, overall decrease with curvature. The analysis was extended to multi-curvature structure and finds significant fluctuation of resonance shift for parallel light incidence. The study can be of profound importance for plasmonic devices using flexible substrates and in fiber-based in vivo applications.

10080-24, Session PMon

Controllable gap plasmon resonance using genetically engineered M13 bacteriophage

Hyerin Song, Heesang Ahn, Dong-Myeong Shin, Kyujung Kim, Pusan National Univ. (Korea, Republic of)

The effective confinement of light in a deep-subwavelength volume can be achieved in metallic nanostructures through the electronic resonance, surface plasmons (SPs). There are few ways to enhance the localization of the field such as adopting metallic nanopost or nanowire structures on the precious metallic film. The achieved highly enhanced field localization through SPs can be exploited for surface-enhanced spectroscopy, biosensor, enhancing energy emitter, and enhanced energy generator. Also, many researches have been tried with few-nanometer gap between the metals for achieving large field enhancements. Still now, the resonances of the plasmonic structures are usually constant and not variable. However, by designing the flexible gap between the metals, we could tune the strong resonance of plasmon at the desired wavelength actively. Here, we propose and demonstrate controllable and even reversible gap using a genetically engineered bacteriophage. The bacteriophage can be chemically bound to gold nanoparticles by genetic engineering process. In this research, we

experimentally and numerically demonstrate the controllable nanoscale gap over a metallic substrate using the bacteriophage. Finally, we could demonstrate the controlling of on-off resonance switch. As changing of the gap between gold nanoparticle and metallic surface, different resonance wavelengths were observed in scattering spectra. The numerical demonstration shows good agreement with our experimental demonstration as well.

10080-25, Session PMon

A uniform gold nanodiscs array fabricated by electron beam lithography and its Raman enhancement study

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For the application of surface enhanced Raman scattering (SERS) measurements, it is believed that an increased in substrates morphology stability would decrease the relative standard deviation (RSD) of SERS intensities. The purpose of this work is to produce substrate with higher morphological stability by utilizing electron beam lithography (EBL) process to fabricate Au nanodiscs array on the glass substrate. Briefly described, with electron beam exposure and developing, the polymethyl methacrylate (PMMA) on the glass became a mask with the periodic pore array. Then, Au was deposited on this PMMA mask. After the lift-off of the Au/PMMA, the Au nanodiscs which were originally in the PMMA pores remained on the glass. The Au nanodisc had a diameter of 100 nm, height of 10 nm and a minimum interval of 25 nm interspacing. Scanning electron microscopy (SEM) and energy disperse X-ray spectrometric (EDS) results showed the Au periodic morphology was well arranged. Surface plasmon resonance at 722 nm and 680 nm indicated this substrate was compatible for red light excitation. The enhancement factor was 4.87 ? 10E6 as tested by dried Rhodamine 6G solution. This EBL Au arrays provide improved stability of Raman enhancement showing an RSD of 5.49%, which is believed to give better results for SERS quantitative analysis, comparing with 22% done by our previous Ag particle deposition substrate. We suppose the similarity between different parts of the array from SEM images may concern with the final RSD of SERS intensities. Further studying is focusing on the computing pattern similarity.

10080-26, Session PMon

Plasmonic lithography according to the thickness of the spacer layer

Taeyeon Kim, Heesang Ahn, Hyerin Song, Kyujung Kim, Pusan National Univ. (Korea, Republic of)

We use the photo lithography in many fields to deal with a fine pattern such as semiconductor, transistor, capacitor, integrated circuit. Light diffraction however makes a fundamental limit on optical resolution. Surface plasmons (SPs) and Extraordinary optical transmission (EOT) have been used to overcome the diffraction limit. SPs are a collective oscillation of electrons generated by the interaction with the entering laser and the free electrons inside the metal. And in the hole of a smaller size than the laser wavelength, EOT is a phenomenon that is transmitted unlike the general optical transmission phenomenon. A lithography method using the SPs and EOT is the plasmonic lithography. Our lithography experiments using a 30W Power, 405 nm wavelength light source demonstrate 250 nm, 300 nm dot array patterns on a 5 um period. The thickness of the mask is 100 nm and consisted of Au (gold). We use Poly(methyl methacrylate) (PMMA) as the spacer layer between the mask and the sample. We have implemented a variety of thickness by adjusting the thickness of the PMMA by spin coating RPM. We demonstrated that the pattern of a unique structure formed according to the thickness. We obtain nano sized hole, rectangular, donut, nano-sucker structure.

10080-27, Session PMon

Switchable optical properties of dyes in DNA origami: gold nanoparticle assemblies

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Optical signals such as fluorescence and Raman scattering generated by a dye near metal nanoparticles can be obtained depending on the distance between the dye and metal surface. DNA origami nanostructures can be used to arrange gold nanoparticles (GNP) and dye molecules with nanometer precision and in well-defined stoichiometry. In this way, the distance dependent signals generated from dye molecules can be studied and dynamic, switchable systems can be created. Fluorescence signals are expected for dyes at large enough metal-dye distances, whereas at very small metal-dye distances fluorescence quenching of the dye is observed, giving rise to surface enhanced Raman scattering (SERS), which is otherwise masked by large fluorescence signals. In order to provide a system where the distance between the dye and the metal nanoparticle can be simply altered, a telomeric DNA sequence modified with a fluorescent dye has been used. This guanine rich, single-stranded telomeric DNA can be folded into a nonduplex structure providing two different distances between the dye and the nanoparticle. The dye-modified telomeric DNA sequence has been positioned on the opposite side of the gold nanoparticle, in relation to the DNA origami structure, and is fluorescent when the telomeric DNA is in its linear form and quenched when the DNA is folded into a guanine quadruplex upon addition of a monovalent cation, bringing the dye close to the metal surface.

10080-28, Session PMon

Plasmonic nanoparticle-based photothermal nanotherapy

Yang Liu, Hsiangkuo Yuan, P. Macaroni, Duke Univ. (United States); Gregory M. Palmer, Duke Univ. School of Medicine (United States); Brant Inman, Tuan Vo-Dinh, Duke Univ. (United States)

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10081-1, Session 1

Re-thinking surface enhance Raman spectroscopy (SERS) sensors with a systems perspective *(Invited Paper)*

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While surface enhanced Raman spectroscopy (SERS) may not compete with the standard central lab approaches for chemical and biological sensing, SERS may have the potential to provide unique capabilities for analytics away from the central lab. Raman spectrometers have evolved from benchtop systems to high-performing handheld instruments that are compatible with analysis of samples in the field. However, for SERS to truly succeed as a "point-of-sample" analytical technique, the SERS sensor must fit the needs of analysis in the field, including little or no sample preparation, minimal peripheral equipment, and ease of use. Traditional plasmonically-active rigid devices do not meet these requirements. Even microfluidic SERS devices generally are not compatible with point-of-sample analysis, as the "world-to-chip" interface presents challenges, and peripheral equipment is generally required.

In this review we will discuss the advances in plasmonic substrates fabricated on porous membranes, leading to SERS sensors that can collect samples via swabbing or dipping, clean up samples through separation, concentrate analytes by lateral flow focusing, and avoid the need for peripheral equipment. In particular, we will focus on inkjet-fabricated devices, which may present the best opportunity for scale-up via roll-to-roll manufacturing. We will also discuss the directions that flexible SERS sensors are moving the field, such as simple fabrication techniques, new support materials, SERS swabs, and SERS-active tapes and films.

10081-2, Session 1

Au particle formation on the electron beam induced nanopre membrane for single molecule analysis

Seong Soo Choi, Myoung Jin Park, Chul Hee Han, Sae-Joong Oh, Tokutaro Yamaguchi, Sun Moon Univ. (Korea, Republic of); Soo Bong Choi, Inchon National University (Korea, Republic of); Yong-Sang Kim, Sungkyunkwan Univ. (Korea, Republic of); Namkyoo Park, Seoul National Univ. (Korea, Republic of)

We will present the Au particle or Au cluster formation on the diffused pore membrane. The Au particle or cluster were formed on the electron beam induced carbon incorporated Au-C membrane. Due to thermodynamic system of the pore surface membrane, the larger Au nanoparticles is getting larger and larger with expense of smaller particles. We also drilled the hole on the diffused membrane. Drilling the hole is observed to be dependent upon the thermal conductivity of the membrane and the beam stability. The formed Au cluster on the membrane with a nanohole can be controlled and utilized as plasmonic nanobio sensor.

10081-3, Session 1

Therapeutic drug monitoring of flucytosine in serum using a SERS-active membrane system

Adam G. Berger, Ian M. White, Univ. of Maryland, College Park (United States)

A need exists for near-real-time therapeutic drug monitoring (TDM), in particular for antibiotics and antifungals in patient serum samples at the point-of-care. To truly fit the point-of-care need, techniques must be rapid and easy to use. Here we report a membrane system utilizing inkjet-fabricated surface enhanced Raman spectroscopy (SERS) sensors that allows sensitive and specific analysis despite the elimination of pipettes, centrifuges, sophisticated chromatography equipment, and other systems relegated to the central lab. We specifically examined the monitoring of flucytosine (5FC), an antifungal, from human serum. We utilized inkjet-fabricated paper SERS sensors as substrates for 5FC detection; the use of paper-based SERS substrates leverages the natural wicking ability and filtering properties of microporous membranes to separate flucytosine spiked in human serum and detect it within clinically relevant ranges. Particularly critical to the success of the sensor is the simple separation of 5FC from the serum components, which foul the plasmonic sensor surface. We investigated the use of microporous membranes and various easy-to-use schemes to allow separation of the flucytosine from human serum. This work establishes a new platform for easy, sensitive, and specific TDM of 5FC from human serum using paper-based SERS sensors and the inherent properties of microporous membranes.

10081-4, Session 1

Plasmonic nanoantenna array with single-chip integrated metal-organic framework for infrared absorption gas sensing

Xinyuan Chong, Ki-Joong Kim, Erwen Li, Yujing Zhang, Oregon State Univ. (United States); Paul R. Ohodnicki Jr., National Energy Technology Lab. (United States) and Carnegie Mellon Univ. (United States); Chih-Hung Chang, Alan X. Wang, Oregon State Univ. (United States)

Surface-enhanced infrared absorption (SEIRA) is a spectroscopic technique used to identify molecular fingerprints by resonant detection of infrared vibrational modes through coupling with the plasmonic modes of metallic nanostructures. Many reported works have demonstrated its capability to enhance the infrared absorption of solid or liquid samples. However, this technique has not been successfully applied to gas sensing yet due to the short light-matter interaction length and intrinsically weak absorption of gas compared to solid or liquid materials. Usually, IR gas sensing is conducted in a gas cell with a long absorption path. In the paper, we propose an integrated photonic device to expand the application of SEIRA to gas sensing by combining metal-organic framework (MOF) ZIF-8 (zeolitic imidazole framework) with plasmonic nanoantenna array. The device consists of an Au nanopatch array on sapphire substrate and is covered by a thin layer of MOF material. The MOF thin film, which is a new class of highly nanoporous material, serves as a gas absorber to selectively adsorb and concentrate CO2 from ambient environment into the thin layer, which has a high spatial overlap with the high intensity optical field of the plasmonic nanopatch antenna array. Namely, we can effectively increase the gas molecule concentration at the hot-spots for the SEIRA device. The experimentally demonstrated peak IR enhancement factor of the device for carbon dioxide sensing is over 1,100 times.

10081-5, Session 1

Surface enhanced Raman scattering in whispering gallery mode microresonators

Steven H. Huang, Xuefeng Jiang, Washington Univ. in St. Louis (United States); Corey Janisch, Alexander S. Cocking, Zhiwen Liu, The Pennsylvania State Univ. (United

States); Lan Yang, Washington Univ. in St. Louis (United States)

In this talk I will discuss surface enhanced Raman scattering in silica microsphere and microtoroid resonators based on whispering gallery mode resonance. Recently silica microspheres have attracted attention as a novel substrate for surface enhanced Raman scattering. Two mechanisms for enhancement have been identified: photonic nano-jet and whispering gallery mode resonance. In most of the previous experiments, however, free space pumping of the microsphere has been used, which has low efficiency in coupling to the whispering gallery modes. In our approach, we use a tapered fiber coupler for a highly efficient coupling to the whispering gallery modes. Coupling to the microresonator is monitored using a tunable laser. We observe both field enhancement and radiation enhancement in the microresonator. Since the linewidth of the whispering gallery modes is much smaller than the linewidth of the Stokes-scattered radiation, signatures of the whispering gallery modes of the resonator are overlaid on top of the Raman spectrum of the material. To demonstrate the system's potential for Raman analysis, we present the whispering gallery mode surface enhanced Raman spectrum of rhodamine 6G thin film coated on a microtoroid resonator.

10081-6, Session 2

Optofluidic reactors for reverse combustion photocatalytic production of hydrocarbons

Perry Schein, David Erickson, Cornell Univ. (United States)

In combustion, hydrocarbon fuels are burned with oxygen to release energy, carbon dioxide and water vapor. Here, we introduce a photocatalytic reactor for reversing this process, when carbon dioxide and water are combined and using optical and thermal energy from the sun hydrocarbons are produced and oxygen is released. This allows for the sustainable production of hydrocarbon products from non-fossil sources, allowing for the development of "green" hydrocarbon products. Our reactors take the form of modular cells of 10 x 10 x 10 cm scale where light is delivered to nanostructured catalysts through the evanescent field around dielectric slab waveguides. The light distribution is optimized through the use of engineered scattering sites to enhance field uniformity. This is combined with integrated fluidic architecture to deliver a stream rich in water and carbon dioxide (such as exhaust from a natural gas burning plant) to the nanostructured catalyst particles in a narrow channel. Exhaust streams rich in oxygen and hydrocarbon products are collected at the outlet of the reactor cell. The cell is heated using solar thermal energy and temperatures of up to 200? C are achieved, enhancing reaction efficiency. Hydrocarbon products produced include methanol as well as other potentially useful molecules for fuel production or precursors to the manufacture of plastics. These reactors can be coupled to solar collectors to take advantage of the sun as a free source of heat and light, and the modular nature of the cells enables scaling to larger deployments.

10081-7, Session 2

Evanescently pumped optofluidic distributed feedback lasers with aqueous gain fluids

Markus Karl, Guy L. Whitworth, Marcel Schubert, Christof P. Dietrich, Ifor D. W. Samuel, Graham A. Turnbull, Malte C. Gather, Univ. of St. Andrews (United Kingdom)

Optofluidic biolasers are an emerging tool for bio-sensing and diagnostics. However, in order to facilitate waveguiding, the most common optofluidic distributed feedback (DFB) laser design relies on high-refractive index gain materials which are usually not biocompatible. We report the realization and characterization of evanescently pumped optofluidic DFB lasers with biocompatible aqueous gain fluids. Record low pump thresholds were achieved by optimizing the mode shape in the waveguide structure. Measuring the photonic band dispersion permits to sense the refractive index of the fluidic gain material. Different biological gain materials were studied on our devices.

10081-8, Session 2

Optofluidic separation of nanoscale bioparticles with plasmonic nanolenses

Xiangchao Zhu, Ahmet Ali Yanik, Univ. of California, Santa Cruz (United States)

Nowadays, optical tweezers are routinely used for trapping and manipulating micrometer size bioparticles in real time. However as particle size becomes smaller the Brownian motion dominates over optical forces. High power lasers are needed to overcome Brownian forces. Use of high power lasers, on the other hand, can cause heating of the bioparticle targets to harmful levels. To overcome this limitation, we propose to use plasmonic nanolenses focusing the incident light in far field region through phase front modulation enabled by a subwavelength thick metasurface. We introduce a nanohole-aperture device that uses a nano-focusing effect to sort bacteria and virus like bioparticles. The patches are patterned on gold-titanium thin film, enabling efficiently coupling of the incident light to the surface plasmons at the metal dielectric interface. The sorting mechanism exploits well controlled balance of scattering, drag, and gravitational forces. At the focus point of a plasmonic nanolens, the laser radiation pressure can be balanced with the drag force (which originates from fluidic flow) to separate microscopic particles with varying characteristics. The size and refractive index of particles are two main factors that determine size-based separation conditions. We demonstrate that the balance between optical and fluidic forces can be adjusted through incident light intensity to selectively sort different sized particles in a dynamic range spanning from 100 nm to 1 ?m.

10081-9, Session 2

Optofluidic lasers with surface gain

Han Zhang, Anirudh Balram, Desheng D. Meng, Yuze A. Sun, The Univ. of Texas at Arlington (United States)

Optofluidic lasers are an emerging technique in recent years and has demonstrated its versatility and unique capability in bio/chemical sensing, molecular diagnostics, and tissue imaging. In this work, we demonstrated optofluidic lasers with a monolayer gain material that self-assembles at the two-phase liquid-liquid interface. The self-assembly process deterministically introduces the gain at the surface of a microdroplet optical cavity, where the lasing mode has maximal interaction with the gain medium. A complete monolayer gain can be achieved in this surface-gain geometry, giving a surface density on the order of 1014 cm-2, which proves to be difficult, if not possible, to achieve in the monolayer gain created at the solid-liquid interface via the surface immobilization method. Microdroplets in the experiment were generated in the microfluidic T-junction and subsequently released onto a superhydrophilic substrate (contact angle = 170°), which supported the microdroplets and allowed them to be individually excited and probed. We demonstrated that the lasing properties and characteristics are of drastic difference between the gain material confined to the liquid-liquid interface (i.e., DiI(3)) and that homogenously distributed in the liquid solution (i.e., Nile Red). We systematically investigated the lasing threshold for different dye concentrations in these two type of lasers. Theoretical analysis was further performed to elucidate the underlying principle. Our study reveals the unique capabilities of the surface-gain geometry optofluidic laser which can be developed into a novel sensing platform to study biophysical and biochemical processes at the molecular level and has vast applications in biomedical diagnostics.

10081-28, Session PSun

Breakthrough in bio sensing: immunodiagnostics using label free and non-specific binding less approach

Divya Sharma, Shoolini Univ. (India); R.P. Dwivedi, Shoolini University (India) and Shoolini University (India)

The expeditious advancement in the bio-sensing detection systems specifically in the area of immunodiagnostics has resulted in many systems which work by using labeled or label free approaches. The labeled approach generally applies complex biomarker molecules which can mark target molecule or the area. This would require a very complex system to analyze further. This is over come by using label free approach, where the real time detection of analyte is performed using the binding agents. Here the main draw back is to specifically control the binding agents to bind on to the target analyte, this results into complex bulky system with the expensive reagents being used. The new frontier is to look beyond the complex labeled biomarkers or the label free binding agents for immunodiagnostics. The novel approach of label free and the Non-Specific binding less is proposed, it uses the synthetic semipermeable membrane or nano filters to filter and isolate the specific target analyte. This approach has an advantage of leaving the analyte intact without any chemical composition changes, there by eliminating the need of cleaning and other binding reagents as in the case of non-specific binding approach. These new non-specific binding less diagnostics can also be performed in real time and provides a low cost solution deployed at the point of care diagnostics.

10081-10, Session 3

Exceptional points enhanced sensing in a whispering-gallery-mode resonator

Weijian Chen, Lan Yang, Washington Univ. in St. Louis (United States)

In this talk, I will discuss the sensitivity enhancement in a whispering-gallery-mode resonator operating at the exceptional points. The exceptional points are obtained by controllably introducing two scatterers within the mode volume of a whispering-gallery-mode resonator. A nanofiber tip is used to mimic a nanoparticle to study the sensitivity enhancement of the resonator operating at the exceptional points. I will present the experimental results demonstrating that the resonator operating at the exceptional points exhibits square-root enhancement in frequency splitting when compared to a single resonator subject to the same amount of perturbation. The dependence of sensitivity enhancement on the angular position and size of the nanofiber tip is also studied. The experimental results agree well with the theoretical predictions and numerical simulations. Our study shows the potential applications of the resonator operating at the exceptional point for ultrasensitive single nanoparticle and biomolecule detection.

10081-11, Session 3

Ring resonator for biosensing via flow-through approach

Romeo Bernini, Immacolata Angelica Grimaldi, Gianluca Persichetti, Genni Testa, Istituto per il Rilevamento Elettromagnetico dell'Ambiente (Italy)

The realization of a simple real time biosensor, in which antibodies are immobilized onto surfaces, represents a promising application in the immunoassay development. Among the various sensing approaches, one of the most promising is based on microring resonators, offering a lot of advantages such as mass production, reduced dimensions, label-free and real time detection. The use of the evanescent field as optical transduction principle allows the development of label-free biosensors, in which the antibody is usually immobilized on the sensor surface and the binding of the antigen can be controlled and followed in real-time.

The overall performances of immunosensors are strongly related to the optimization of the immobilization process and the integration between the microfluidic parts and the optical detection system. The combination of these two aspects makes the biosensing process very efficient, with a consequent reduction of the response time and improvement of the immobilization process efficiency.

In this work we explore the working mechanism of a flow-through microresonator platform. A drilled hole, in the center of the ring, allows the active transport mechanism of the analyte toward the sensing surface with a consequent reduction of the response time. Moreover, we study the effects of oxygen plasma, in terms of duration times and plasma power, on immobilization efficiency of immunoglobulin G (IgG). An improvement of about 20% of the protein adsorption is ascribed to chemico-physical modification of SU-8. The measured sensor response time in flow-through configuration is about five times shorter respect to standard flow-over configuration.

10081-12, Session 3

Resonant photonic structures in porous silicon for biosensing

Sharon M. Weiss, Gilberto A. Rodriguez, Yiliang Zhao, Tengfei Cao, Vanderbilt Univ. (United States); Yasmin M. Graham, Vanderbilt Univ. (United States) and Univ. of Maryland, Baltimore County (United States); Girija Gaur, Vanderbilt Univ. (United States)

The formation of resonant photonic structures in porous silicon leverages the advantage of high surface area for improved molecular capture that is characteristic of porous materials with the advantage of high detection sensitivity that is a characteristic of resonant optical devices. This talk will provide an overview of the biosensing capabilities of a variety of resonant porous silicon photonic structures including ring resonators, photonic crystal nanobeams, Bloch surface waves, annular Bragg resonators, and microcavities. Detection sensitivities >1000 nm/RIU are reported for small molecule detection. The challenge of detecting molecules that approach and exceed the pore diameter will also be addressed.

10081-13, Session 3

Whispering-gallery-mode resonators and their applications for nanoscale sensing and measurement *(Invited Paper)*

Steven H. Huang, Weijian Chen, Bo Peng, Guangming Zhao, Huzeyfe Yilmaz, Xiangyi Xu, Xuefeng Jiang, Sahin Kaya Özdemir, Lan Yang, Washington Univ. in St. Louis (United States)

Light-matter interactions are the fundamental basis for many phenomena and processes in optical devices. In this talk, I will introduce and explain ultra-high-quality (Q) optical Whispering-Gallery-Mode (WGM) microresonators, in which light-matter interactions are significantly enhanced due to their superior capability to trap light field in a highly confined volume with low loss. WGM resonators have shown great promise for a variety of fields of science, spanning from on-chip microresonator lasers to nonlinear optics and ultra-sensitive label-free sensing. After briefly introducing the physical concepts of WGM microresonators and their coupling with a microfiber waveguide, I will discuss ultra-high-Q microresonators and microlasers for ultra-sensitive self-referencing detection and sizing of single virions, dielectric and metallic nanoparticles. These recent advancements in WGM microresonators will enable a new class

of ultra-sensitive and low-power sensors for investigating the properties and kinetic behaviors of nanomaterials, nanostructures, and nanoscale phenomena. Afterward, I will discuss our recent exploration of fundamental physics, such as parity-time symmetry and light-matter interactions around exceptional points (EPs), in high-quality WGM resonators, which can be used to enhance weak signals in sensing applications. In the end, I will present a new generic and hand-held microresonator platform that was transformed from a table-top setup, which will help release the power of high-Q WGM resonator technologies.

10081-14, Session 4

On-chip infrared sensors: redefining the benefits of scaling *(Invited Paper)*

Derek Kita, Hongtao Lin, Tian Gu, Anuradha M. Agarwal, Massachusetts Institute of Technology (United States); Anupama Yadav, Kathleen A. Richardson, CREOL, The College of Optics and Photonics, Univ. of Central Florida (United States); Igor Luzinov, Clemson Univ. (United States); Juejun Hu, Massachusetts Institute of Technology (United States)

Infrared (IR) spectroscopy is widely recognized as a gold standard technique for chemical and biological analysis. Traditional IR spectroscopy relies on fragile bench-top instruments located in dedicated laboratory settings, and is thus not suitable for emerging field-deployed applications such as in-line industrial process control, environmental monitoring, and point-of-care diagnosis. Recent strides in photonic integration technologies provide a promising route towards enabling miniaturized, rugged platforms for IR spectroscopic analysis. It is therefore attempting to simply replace the bulky discrete optical elements used in conventional IR spectroscopy with their on-chip counterparts. This size down-scaling approach, however, cripples the system performance as both the sensitivity of spectroscopic sensors and spectral resolution of spectrometers scale with optical path length.

In light of this challenge, we will discuss two novel photonic device designs uniquely capable of reaping performance benefits from microphotonic scaling. We leverage strong optical and thermal confinement in judiciously designed micro-cavities to circumvent the thermal diffusion and optical diffraction limits in conventional photothermal sensors and achieve a record 104 photothermal sensitivity enhancement. In the second example, an on-chip spectrometer design with the Fellgett's advantage is analyzed. The design enables sub-nm spectral resolution on a millimeter-sized, fully packaged chip without moving parts.

10081-15, Session 4

Enhanced surface sensitivity in microring resonator biosensor based on subwavelength grating waveguides

Hai Yan, The Univ. of Texas at Austin (United States); Lijun Huang, The Univ. of Texas at Austin (United States) and Beijing Univ. of Posts and Telecommunications (China); Xiaochuan Xu, Naimei Tang, Swapnajit Chakravarty, Omega Optics, Inc. (United States); Ray T. Chen, The Univ. of Texas at Austin (United States) and Omega Optics, Inc. (United States)

Microring resonators on silicon-on-insulator substrate has been demonstrated to be promising in sensing applications, where biomolecule layers immobilized on the surface of the microring induces resonance shift in the transmission spectrum. However, this type of evanescent wave sensing faces limitation in surface sensing: the sensitivity drops rapidly with increasing thickness of the biomolecule layer accumulated on the sensor surface. Here we propose a microring resonator biosensor based on a novel subwavelength grating (SWG) waveguide structure. The SWG waveguide consists of periodic silicon pillars in the propagation direction with a period (200-250nm) much smaller than the operating wavelength of around 1550nm. In this proposed structure, effective sensing region includes not only the top and side of the waveguide, where evanescent wave exists, but also the space in between the silicon pillars which is on the path of the propagation mode. This leads to greatly increased bulk refractive index sensitivity as well as extended surface sensing region with constantly high surface sensitivity. Simulation shows that the surface sensitivity (resonance shift / surface layer thickness) remain almost constant in the first 20-40nm thick layer upon the surface. In experimental demonstration, microring resonator biosensors based on both conventional channel waveguides and SWG waveguides are fabricated. Special tuning of the pillar shape in the SWG is utilized to minimize the bending loss in the SWG microring. A comparison in the sensing test between the two types of sensors demonstrates the superior surface sensing capability in the proposed SWG microring sensor.

10081-16, Session 4

Nanoparticle trapping and characterization with open microcavities

Aurelien Trichet, Philip R. Dolan, Dean James, Gareth M. Hughes, Claire Vallance, Jason M. Smith, Univ. of Oxford (United Kingdom)

Thanks to their low mode volume and high finesse, optical microresonators have emerged as a promising avenue to detect and measure properties of single nanoparticles such as viruses or gold nanoparticles. Thanks to the resulting electromagnetic field enhancement, small nanoparticles, viruses and even single proteins have been trapped in hollow resonators such as photonic crystals or plasmonic tweezers. Such trapping devices with sensing capabilities are on the verge of finding powerful applications in interdisciplinary science. However, the quest for a candidate bringing together in-situ detection, trapping and multiple quantitative measurements of the particle properties supported by a comprehensive understanding still remain elusive.

In this work, we show that open-access microcavities fulfil these criteria. Such resonators are made up of two micro-mirrors facing each other separated by a fluid medium in which nanoparticles can diffuse. We have recorded the cavity mode spectra while nanoparticles were optically trapped. Our results demonstrate that these microcavities can be used as optical tweezers with in-situ force calibration and nanoparticle sensing capabilities, including measurement of shape anisotropy. The shift in cavity mode wavelength during a trapping event provides information on both the nanoparticle and trap properties, as well as on the trapping force holding the particle in the trap. We are able to determine in real-time the nanoparticle polarizability, i.e. its optical response to an electromagnetic field, its coefficient of friction and characterize its shape anisotropy. The high level of control in this device makes it a robust analytical tool for real-time nanoparticle characterisation and monitoring.

10081-17, Session 4

Towards a manufacturing ecosystem for integrated photonic sensors

Benjamin L. Miller, Univ. of Rochester Medical Ctr. (United States)

Laboratory-scale demonstrations of optical biosensing employing structures compatible with CMOS fabrication, including waveguides, Mach-Zehnder interferometers, ring resonators, and photonic crystals, have provided ample validation of the promise of these technologies. However, to date there are relatively few examples of integrated photonic biosensors in the commercial sphere. The lack of successful translation from the laboratory

to the marketplace is due in part to a lack of robust manufacturing processes for integrated photonics overall. This talk will describe efforts within the American Institute for Manufacturing Photonics (AIM Photonics), a public-private consortium funded by the Department of Defense, State governments, Universities, and Corporate partners to accelerate manufacturing of integrated photonic sensors.

10081-18, Session 4

Reducing user error in dipstick urinalysis with a low-cost slipping manifold and mobile phone platform *(Invited Paper)*

Gennifer T. Smith, Nicholas Dwork, Saara A. Khan, Matthew Millet, Kiran Magar, Stanford Univ. (United States); Mehdi Javanmard, Rutgers, The State Univ. of New Jersey (United States); Audrey K. Bowden, Stanford Univ. (United States)

Urinalysis dipsticks were designed to revolutionize urine-based medical diagnosis. They are cheap, extremely portable, and have multiple assays patterned on a single platform. They were also meant to be incredibly easy to use. Unfortunately, there are many aspects in both the preparation and the analysis of the dipsticks that are plagued by user error. This high error is one reason that dipsticks have failed to flourish in both the at-home market and in low-resource settings. Sources of error include: inaccurate volume deposition, varying lighting conditions, inconsistent timing measurements, and misinterpreted color comparisons. We introduce a novel manifold and companion software for dipstick urinalysis that eliminates the aforementioned error sources. A micro-volume slipping manifold ensures precise sample delivery, an opaque acrylic box guarantees consistent lighting conditions, a simple sticker-based timing mechanism maintains accurate timing, and custom software that processes video data captured by a mobile phone ensures proper color comparisons. We show that the results obtained with the proposed device are as accurate and consistent as a properly executed dip-and-wipe method, the industry gold-standard, suggesting the potential for this strategy to enable confident urinalysis testing. Furthermore, the proposed all-acrylic slipping manifold is reusable and low in cost, making it a potential solution for at-home users and low-resource settings.

10081-19, Session 5

X-ray excited luminescent chemical imaging (XELCI) for non-invasive imaging of implant infections *(Invited Paper)*

Jeffrey Anker, Clemson Univ. (United States) and COMSET (United States); Unaiza Uzair, Donald W. Benza, Tzuen-Rong J. Tzeng, Yash S. Raval, Clemson Univ. (United States); Caleb J. Behrend, Virginia Tech Carilion School of Medicine (United States)

Bacteria can colonize on the surface of implanted medical devices and form biofilms that are resistant to antibiotics and the host's immune system. Unfortunately these implant infections are difficult to monitor, especially at early stages or during antibiotic treatment when the surviving bacteria are localized at the implant surface. We are developing an optically-based pH indicator film that can be coated onto implant surfaces to enable imaging via X-ray excited luminescencent chemical imaging (XELCI). XELCI is a type of scanning optical microscopy wherein a narrow X-ray beam irradiates a radioluminescent film on the implant surface creating a local luminescent spot with pH -dependent spectra. Although the luminescence scatters and blurs as it passes through the tissue in the "far field," the spectrum depends upon the local pH at the luminescence source, and image resolution is defined by the X-ray beam width. We demonstrate the ability to measure pH and image changes during bacterial growth and antibiotic treatment through ex-vivo tissue slices.

10081-20, Session 5

Order of magnitude classification of bacterial solutions

Rachel Guo, Cushla McGoverin, Simon Swift, Frédérique Vanholsbeeck, The Univ. of Auckland (New Zealand)

In many fields of microbiological study enumeration of bacteria is vital. Traditionally this has involved standard plate counts, which require sample serial dilution, inoculation of solid growth media and incubation overnight or longer. More rapid methods are desirable, however, there is the caveat that these methods should not substantially increase testing costs. We have investigated using the fluorescence spectra collected from acridine orange (AO) stained solutions of bacteria to determine bacterial concentration. Escherichia coli ATCC 25922 samples spanning the concentration range of 0 - 108 cells/ml were stained with AO and washed. Three sample preparations were investigated: 0.2% w/v AO followed by 3 washing cycles; 0.02% w/v AO and 2 washing cycles; 0.002% w/v AO and no washing cycles. A fibre-based spectrometer was used to measure fluorescence spectra from these samples. The intensity of fluorescence from bound AO was not linearly correlated with bacterial concentration. However, fluorescence signals were reproducible for AO stain concentration - bacterial concentration pairs. The spectral data set collected was subsequently analysed using independent components analysis. From these analyses classification models were calculated to group samples by the order of magnitude of bacterial concentration. Using the 0.2% and 0.02% w/v AO sample preparations it was possible to rapidly classify the order of magnitude of bacterial concentration for samples with bacterial concentrations above 105 CFU/ml. This rapid and relatively inexpensive method for bacterial enumeration by order of magnitude will reduce time and cost of microbiological testing when gross concentration information is required.

10081-21, Session 5

SYTO 9 staining of bacteria

Cushla McGoverin, The Univ. of Auckland (New Zealand); Scott Choi, Craig Tuffnell, Veritide Ltd. (New Zealand); Julia Robertson, Simon Swift, Frédérique Vanholsbeeck, The Univ. of Auckland (New Zealand)

The microbiological integrity of food products is a major concern in supply chains. Current assessment is largely reliant on culturing samples on solid growth media; a process which is laborious and requires at least an overnight time span. We are developing a near-real time, user-friendly method for determine bacterial count in samples taken within beef processing supply chains.

Central to this method is a robust fibre-based fluorimeter, the optrode. This systems consists of a 473 nm laser for excitation; a DAQ controlled shutter ensures the sample is only illuminated during fluorescence measurement, thereby reducing experimental variation induced by photobleaching. Using a 2x2 coupler laser light irradiates the sample and a photodiode, this photodiode measurement allows incident intensity of laser light during measurement to be monitored. An Ocean Optics 65000 CCD spectrometer is used to collect fluorescence spectra from 400-800 nm.

The bacteria of interest are often not natively fluorescent at 473 nm, we therefore use a nucleic acid stain, SYTO 9, to fluorescently dye bacterial cells. We have been assessing staining parameters such as time of stain to ensure maximum fluorescence signal is obtained in the minimum amount of time. In addition we have assessed the difference in stain uptake between Gram positive and Gram negative bacterial cells.

10081-22, Session 5

Measuring micron-scale displacements through tissue using luminescent spectral rulers for monitoring bone healing

Jeffrey Anker, Clemson Univ. (United States) and SC BioCRAFT (United States) and COMSET (United States); Melissa M. Rogalski, Donald W. Benza, Hunter L. Pelham, Fathima S. Ameer, John D. DesJardins, Clemson Univ. (United States); Caleb J. Behrend, Virginia Tech Carilion School of Medicine (United States)

There are approximately 2 million fracture fixation surgeries performed per year in the US. The time required for patients to heal before resuming bearing weight directly affects their mobility, rehabilitation costs, and productivity. In addition, approximately 100,000 injuries (5% of fixation surgeries) go on to non-union which require additional surgeries to correct. A similar amount also become infected, which delays healing and may also cause implant loosening. Measuring strain under load in vivo is critical for assessing bone healing by determining the load-sharing between implanted fixation devices and healing bone fracture. We describe luminescent spectral rulers to non-invasively measure mechanical strain and fracture displacement through tissue. These spectral rulers contain two overlaid substrates: an encoder substrate patterned with alternating stripes of luminescent materials, and a mask containing opaque regions and transparent windows. Displacement of the encoder with respect to the mask modulates which luminescent material is visible through the transparent regions of the mask; the displacement can therefore be inferred from the spectrum. We measured luminescence spectral ratio as a function of position for patterns made from fluorescent quantum-dots, X-ray scintillators, and upconversion phosphors. Using 500 μm thick encoder lines, the noise level measured through tissue was equivalent to 2-3 μm uncertainty shot noise was the main source of noise. Work is ongoing to apply these sensors to non-invasively measure tension and bending in orthopedic screws and plates.

10081-23, Session 6

Peptide-based antibody alternatives for biological sensing in austere environments *(Invited Paper)*

Matthew B. Coppock, Deborah A. Sarkes, Margaret M. Hurley, Dimitra N. Stratis-Cullum, U.S. Army Research Lab. (United States)

The most critical component of a biosensor, the biorecognition element, must exhibit a high selectivity and strong affinity for a target of interest in operational sensing. Monoclonal antibodies are the current standard reagents for such devices, but their adaptability, manufacturability, and stability greatly limit their effectiveness in fieldable sensors. Peptides have emerged as potential antibody replacements in such applications due to their similar binding performance, extreme chemical and thermal stabilities, and on-demand scalability. In conjunction with modeling capabilities, work at the Army Research Lab focuses on protein catalyzed capture (PCC) agent technology and bacterial display for the discovery of these novel peptide binding reagents. The synthetic, bottom-up PCC agent technology uses an iterative, in situ “click chemistry” approach to produce high performing peptides against specific epitopes translatable to the protein target. Bacterial display allows rapid reagent discovery due to the combination of fast bacterial growth and effective peptide sequence enrichment through multiple rounds of biopanning. Recent advances in both methods will be highlighted in regards to the discovery of reagents against Army high priority protein targets for soldier safety, performance, and diagnostics.

10081-24, Session 6

Development of terahertz otoscope for diagnosing otitis media

Tae-In Jeon, Korea Maritime and Ocean Univ. (Korea, Republic of); Young Bin Ji, Yonsei Univ. College of Medicine (Korea, Republic of); Hyeon Sang Bark, Korea Maritime and Ocean Univ. (Korea, Republic of); Sam Kyu Noh, Korea Research Institute of Standards and Science (Korea, Republic of); Seung Jae Oh, Yonsei Univ. College of Medicine (Korea, Republic of)

A novel terahertz (THz) otoscope is designed and fabricated to help physicians to diagnose otitis media (OM) with both THz diagnostics and conventional optical diagnostics. The inclusion of indium tin oxide (ITO) glass in the THz otoscope allows physicians to diagnose OM with both THz and conventional optical diagnostics. To determine THz diagnostics for OM, we observed reflection signals from samples behind a thin dielectric film and found that the presence of water behind the membrane could be distinguished based on THz pulse shape. We verified the potential of this tool for diagnosing OM using mouse skin tissue and a human tympanic membrane samples prior to clinical application. The presence of water absorbed by the human membrane was easily distinguished based on differences in pulse shapes and peak-to-peak amplitudes of reflected THz pulses. The potential for early OM diagnosis using the THz otoscope was confirmed by alteration of THz pulse depending on water absorption level.

10081-25, Session 6

Accurate live and dead bacterial cell enumeration using flow cytometry

Fang Ou, Cushla McGoverin, Simon Swift, Frédérique Vanholsbeeck, The Univ. of Auckland (New Zealand)

Flow cytometry (FCM) is based on the detection of scattered light and fluorescence to identify cells with particular characteristics of interest. However most FCM cannot precisely control the flow through its interrogation point and hence the volume and concentration of the sample cannot be immediately obtained. The easiest, most reliable and inexpensive way of obtaining absolute counts with FCM is by using reference beads. We investigated a method of using FCM with reference beads to measure live and dead bacterial concentration over the range of 106 to 108 cells/mL and ratio varying from 0 to 100%. We believe we are the first to use this method for such a large cell concentration range while also establishing the effect of varying the live:dead bacteria ratios.

Escherichia coli solutions with differing ratios of live:dead cells were stained with fluorescent dyes SYTO 9 and propidium iodide (PI), which label live and dead cells, respectively. Samples were measured using a LSR II Flow Cytometer (BD Biosciences); using 488 nm excitation with 20 mW power. Both SYTO 9 and PI fluorescence were collected and threshold was set to side scatter. Traditional culture-based plate count was done in parallel to the FCM analysis. The concentration of live bacteria from FCM was compared to that obtained by plate counts. Preliminary results show that the concentration of live bacteria obtained by FCM and plate counts correlate well with each other and indicates this may be extended to a wider concentration range or for studying other cell characteristics.

10081-26, Session 6

Light assisted drying (LAD) for protein stabilization: optimization of laser processing parameters

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The goal of this project is to develop a new light-assisted processing method to dehydrate protein-based biologics in preparation for long-term storage. Indicator proteins are used in a variety of diagnostic assays ranging from high-throughput 96-well plates to new microfluidic devices. A challenge in the development of protein-based assays is preserving the structure of the protein during production and storage of the assay, as the structure of the protein is responsible for its functional activity. Freeze-drying or freezing are currently the standard for the preservation of proteins, but these methods are expensive and can be challenging in some environments due to a lack of available infrastructure. An inexpensive, simple processing method that enables supra-zero temperature storage of proteins used in assays is needed. Light-assisted drying offers a relatively inexpensive method for drying samples. Proteins suspended in a trehalose solution are dehydrated using near-infrared laser light. The laser radiation speeds drying and as water is removed the sugar forms a protective matrix. The goal of this study is to determine processing parameters that result in fast processing times and low end moisture contents (EMC) while maintaining the functionality of embedded proteins. We compare the effect of changing processing wavelength, power and resulting sample temperature, and substrate material on the EMC for two NIR laser sources (Nd:YAG, 1064 nm and Thulium fiber, 1850 nm). The 1850 nm laser resulted in the lowest EMC (0.1836±0.09 gH2O/gDryWeight) after 10 minutes of processing on borosilicate glass microfiber paper. This suggests a storage temperature of ~3°C.

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High-throughput screening based on label-free detection of small molecule microarray

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We present a novel high-throughput screening platform based on small-molecule microarrays (SMMs) and label-free oblique-incidence reflectivity difference (OI-RD) microscope. Firstly, we developed OI-RD microscope which is a microarray compatible label-free high-throughput detection method. OI-RD is the special form of an ellipsometry measuring the phase change of the reflected beam which is proportional to the surface mass density of biomolecules on surface so that OI-RD enables label-free detection of biomolecular interactions, getting rid of problems associated with labeling. Compatible with microarray, OI-RD is able to monitor over 15,000 interactions in a single experiment, providing a novel platform for high-throughput screening. Secondly, we developed isocyanate chemistry to prepared SMMs which is able to immobilize compounds with any nucleophilic residue on phenyl-isocyanate functionalized glass slides with high efficiencies. By printing 3,375 compounds on phenyl-isocyanate functionalized glass slides followed by 45 deg post-printing annealing of SMM, over 73% compounds can be successfully immobilized on surface. Based on combination of label-free OI-RD microscope and as-prepared SMMs, we screened some non-labeled target proteins in high-throughput and label-free mode and we found hits for respective target protein. The novel high-throughput screening platform enables target proteins with unknown structure and/or unknown function to be effectively screened. In addition, the advantages, low sample consumption, high sensitivity, and multi-target screening, make it have wide applications in high-throughput screening.