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1–6 February 2014

Exhibition
BiOS Expo: 1–2 February
Photonics West: 4–6 February

Location
The Moscone Center
San Francisco, California, USA

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SPIE Photonics West

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Contents

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Conference 8926A: Photonics in Dermatology and Plastic Surgery

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8926 Photonic Therapeutics and Diagnostics X

8926-1, Session Key

Assessing the colors of human skin *(Keynote Presentation)*

Nikiforos Kollias, Consultant (United States) and Univ. of British Columbia (Canada)

The color of human skin has been studied through reflectance methods with either colorimetric approaches or with spectrophotometric approaches. Colorimetric methods measure color in a psychophotometric approach, the end products is the quantification of human perception. In reflectance spectrophotometric methods the goal is to estimate the concentration of the contributing chromophores to the appearance of the skin. Both methods have made a great deal of progress and yet there remain a number of shortcomings. These become evident when we study large populations including imaging of extended areas of skin and try to catalogue subjects in terms of their color parameters. There is an additional dimension to color assessment and that has to do with the way the human visual system processes visual information - this becomes particularly noticeable when multiple colors are perceived in close proximity to each other. The other dimension of color in art or in skin assessment is the elicitation of emotions - “the psycho”- in the colorimetric scales.

8926-2, Session 1

Transparent polymer-based oxygen sensing wound dressing

Zongxi Li, Emmanouil Rousakis, Conor L. Evans, Massachusetts General Hospital (United States)

Novel polymer-based oxygen sensing wound dressings were developed for the therapeutic monitoring of chronic wounds such as burns, flaps and skin grafts, where tissue oxygenation plays an important role in the patient’s prognosis. The dressings, fabricated from polydimethylsiloxane or liquid bandage materials, contain an oxygen-sensing phosphor and a reference dye for the visualization of skin oxygenation in a non-invasive fashion. To provide a burst of illumination to capture the emission from the sensing bandage, a custom camera equipped with a flash unit was used as a detection system. A delay-trigger mechanism was introduced, which allows the oxygen-dependent phosphorescence of the wound dressing to be selectively captured even when used on highly auto-fluorescent skin, producing images with excellent signal-to-noise ratio. Using this camera system, the emission of the two dyes can be used to build a quantitative colorimetric map of tissue O2. This novel design has the advantage of providing a continuous two-dimensional map of the tissue oxygenation level at physiological conditions, which cannot be achieved by existing clinical tissue oxygen measurement approaches. The dressing senses tissue oxygenation through intact skin, providing information on wound perfusion and healing potential non-invasively. The real-time tissue oxygenation status obtained can be used to assess the patient’s risk of deterioration and wound healing potentials, while the oxygenation level over time can be used to monitor healing progress and treatment response. The dressings have been tested on an ischemic flap model on living swine to demonstrate their oxygen sensing capabilities.

8926-3, Session 1

Irradiation with EMOLED improves the healing process in superficial skin wounds

Riccardo Cicchi, Istituto Nazionale di Ottica (Italy); Francesca Rossi, Francesca Tatini, Istituto di Fisica Applicata Nello Carrara (Italy); Stefano Bacci, Gaetano De Siena, Univ. degli Studi di Firenze (Italy); Domenico Alfieri, Light4Tech Firenze S.r.l. (Italy); Roberto Pini, Istituto di Fisica Applicata Nello Carrara (Italy); Francesco S. Pavone, European Lab. for Non-linear Spectroscopy (Italy)

A faster healing process was observed in superficial skin wounds after irradiation with the EMOLED photocoagulator. The instrument consists of a compact handheld photocoagulation device, useful for inducing coagulation in superficial abrasions. The illumination is provided by a high power blue LED. Blue light is selectively absorbed by haemoglobin and converted into heat through a photothermal effect. In this study, 10 Sprague Dawley rats were mechanically abraded in four regions of their back: two regions were used as a control and the other two were treated with EMOLED. The photothermal effect was monitored by an infrared thermocamera in order to avoid accidental thermal damage. Visual observations, histopathological analysis and non-linear microscopic imaging performed after 8 days from the treatment showed no adverse reactions and no thermal damage in both treated areas and surrounding tissues. Moreover, a faster healing process and a better-recovered morphology was evidenced in the treated tissue with respect to the untreated tissue. Compared to the control regions, a reduced inflammatory response, a higher collagen content, and a skin morphology more similar to normal skin were observed in the treated regions. Collagen organization in the two regions was characterized using image pattern analysis algorithms on SHG images, demonstrating a fully recovered aspect of dermis as well as a faster neocollagenesis in the treated regions. This study demonstrates that the selective photothermal effect we used for inducing immediate coagulation in superficial wounds is associated to a minimal inflammatory response, which provides reduced recovery times and improved healing process.

8926-4, Session 1

Quantitative assessment of graded burn wounds in a porcine model using spatial frequency domain imaging (SFDI) and laser speckle imaging (LSI)

Adrien Ponticorvo, Bruce Yang, Beckman Laser Institute and Medical Clinic (United States); David M. Burmeister, Robert J. Christy, U.S. Army Institute of Surgical Research (United States); Bernard Choi, Anthony J. Durkin, Beckman Laser Institute and Medical Clinic (United States)

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, it is difficult to diagnose burns that fall between these extremes. Because of this, treatment options are often delayed and run the risk of being less effective. Here we investigated spatial frequency domain imaging (SFDI) and laser speckle imaging (LSI) as non-invasive technologies to predict burn severity. By projecting patterned illumination onto the tissue surface, SFDI is able to quantify the scattering coefficients as

well as absolute concentration changes of hemoglobin. LSI utilizes a projected speckle pattern to extrapolate blood flow measurements. We have used SFDI/LSI to study controlled burn wounds of graded severity in a Yorkshire pig model. The burn wounds were monitored for five days and burn depths were verified using histological analysis. We found that monitoring changes in optical properties and blood flow has the potential to predict burn severity. Deeper burns corresponded with a reduction in the scattering coefficient, while increase scattering was observed in more superficial burns. While all burns showed a decrease in blood flow and an increase in deoxygenated hemoglobin, deeper burns showed a greater decrease in blood flow and a greater increase in deoxygenated hemoglobin compared to more superficial burns. Investigating these parameters has the potential to help in predicting burn severity and also monitoring tissue healing, which would be critical for evaluating the efficacy of different treatment options.

8926-5, Session 1

Prognostic prospective of laser induced fluorescence as an objective tool to evaluate collagen deposition in thermal wounds: an ex vivo study

Vijendra Prabhu, Anusha Acharya, Satish Bola Sadashiva Rao, Pramod Kumar, Anurag Sharan, Krishna K. Mahato, Manipal Univ. (India)

Thermal injuries are considered as the most frequent and dreadful forms of trauma. Quick and objective assessment of treatment regimen plays vital role in controlling morbidity and mortality. In the present work, we aimed to investigate the predicative potential of laser induced fluorescence (LIF) in assessing collagen synthesis during the wound healing progression following thermal injury. To this end, granulation tissues of thermal wounds before grafting were collected from the patients of age group 18- 60 years with prior consent. LIF measurements of the wound granulation tissues were carried out by exciting them with 325 nm He-Cd laser (2 mW power). Collagen intensity with respect to normalized NADH levels in each of the samples was computed and tabulated. In addition, biochemical estimation of the hydroxyproline was also carried out from the same granulation tissues. Spectroscopic assessment of the wound granulation tissues displayed varied collagen levels indicating different degree of healing. Likewise, biochemical estimation of granulation tissue exhibited a wide range of hydroxyproline levels. The findings of the present ex vivo study suggest that both the techniques are equally informative in evaluating collagen content in tissues. However, with LIF, it is more advantageous in the sense that it is fast, objective, cost effective as well as non–destructive method. Thus, it can be concluded that laser induced fluorescence has the potential to be used as an objective tool to qualitatively determine the collagen levels in wound granulation tissues and provide information helpful for making clinical decisions

8926-6, Session 1

Monitoring the influence of compression therapy on pathophysiology and structure of a swine scar model using multispectral imaging system

Pejhman Ghassemi, The Catholic Univ. of America (United States) and MedStar Health Research Institute (United States); Taryn E. Travis, Lauren T. Moffatt, MedStar Health Research Institute (United States); Jeffrey W. Shupp, MedStar Health Research Institute (United States) and The Catholic Univ. of America (United States); Jessica C. Ramella-Roman, The Catholic Univ. of America (United States) and MedStar Health Research Institute (United States)

Scar contractures can lead to significant reduction in function and inhibit patients from returning to work, participating in leisure activities and even render them unable to provide care for themselves. In addition these patients suffer great psycho-social setbacks due to their disfigured appearance. Compression therapy has long been a standard treatment for scar prevention but due to the lack of quantifiable metrics of scar formation scant evidence exists of its efficacy. There is a continued need for better treatment and assessment techniques to treat and diagnose patients with scars. The Vancouver Scar Scale (VSS) was introduced in the nineties and relies on the physician subjective evaluation of skin pliability, height, vascularity, and pigmentation. Based on VSS, we have recently introduced a multispectral imaging system to quantify pathophysiology (hemoglobin, blood oxygenation, melanin, etc) and structural features (roughness and collagen matrix) of scar. In this study, hypertrophic scars are monitored in-vivo in a porcine model using the imaging system to investigate influence of compression therapy on its quality. Briefly, dermal wounds are created in a porcine model and allowed to form into scar. To perform compression therapy, an automatic pressure delivery system is designed and constructed to create and maintain constant level(s) of pressure on the scar. Scars with and without compression therapy are assessed at various time periods using the imaging system. To demonstrate our hypothesis, we utilize the imaging system to quantify the extent of abnormal restructuring in a well-controlled scar. This includes changes in skin roughness, changes in optical properties, and changes in the dermis collagen matrix.

8926-7, Session 1

Hyperspectral characterization of an in vitro wound model

Lise L. Randeberg, Janne-Lise Hegstad, Lukasz A. Paluchowski, Norwegian Univ. of Science and Technology (Norway); Brita S. Pukstad, Norwegian Univ. of Science and Technology (Norway) and St. Olavs Hospital (Norway)

Wound healing is a complex process not fully understood. There is a need of better methods to evaluate the different stages of healing, and optical characterization is a promising tool in this respect.

In this study hyperspectral imaging was employed to characterize an in vitro wound model.

The wound model was established by first cutting circular patches of human abdominal skin using an 8mm punch biopsy tool, and then creating dermal wounds in the center of the skin patches using a 5mm tool. The wounds were incubated in medium with 10% serum and antibiotics. Hyperspectral images were collected every three days using a push broom hyper spectral camera (Hyspex VNIR1600). The camera had a spectral resolution of 3.7 nm and was fitted with a close up lens giving a FOV of 2.5 cm and a spatial resolution of 29 micrometer. Samples for histology were collected throughout the measurement period, which was 21 days in total. Data were processed in ENVI and Matlab.

A successful classification based on hyperspectral imaging of the implemented model is presented. It was not possible to see the healing zone in the in vitro model with the naked eye without dying. The hyperspectral results showed that newly formed epithelium could be imaged without any additional contrast agents or dyes. It was also possible to detect non-viable tissue. In vitro wound models and hyperspectral imaging can thus be employed to gain further insight in the complicated process of healing in different kinds of wounds.

8926-8, Session 2

Imaging guided photothermolysis through two-photon absorption demonstrated on mouse skin: a potential tool for highly targeted skin treatment

Hequn Wang, The BC Cancer Agency Research Ctr. (Canada); Soodabeh Zandi, The Univ. of British Columbia (Canada); Anthony M. Lee, Jianhua Zhao, The BC Cancer Agency Research Ctr. (Canada); Harvey Lui M.D., David I. McLean M.D., The Univ. of British Columbia (Canada); Haishan Zeng, The BC Cancer Agency Research Ctr. (Canada) and Univ. of British Columbia (Canada)

One-photon absorption based traditional laser treatment may not necessarily be selective at the microscopic level, thus could result in un-intended tissue damage. Our objective is to test whether two-photon absorption (TPA) could provide highly targeted tissue alteration of specific region of interest without damaging surrounding tissues. TPA based laser treatments (785 nm, 140 fs pulse width, 90 MHz) were performed on ex vivo mouse skin using different average power levels and irradiation times. Reflectance confocal microscopy (RCM) and combined second- harmonic-generation (SHG) and two-photon fluorescence (TPF) imaging channels were used to image before, during, and after each laser treatment. The skin was fixed, sectioned and H&E stained after each experiment for histological assessment of tissue alterations and for comparison with the non-invasive imaging assessments. Localized destruction of dermal fibers was observed without discernible epidermal damage on both RCM and SHG+TPF images for all the experiments. RCM and SHG+TPF images correlated well with conventional histological examination. This work demonstrated that TPA-based light treatment provides highly localized intradermal tissue alteration. With further studies on optimizing laser treatment parameters, this two photon absorption photothermolysis method could potentially be applied in clinical dermatology.

8926-9, Session 2

Fractional laser technologies and applications in dermatology *(Invited Paper)*

Dieter Manstein, Wellman Ctr. for Photomedicine (United States)

No Abstract Available

8926-10, Session 2

Heat profiles in laser irradiated nails

Uwe Paasch, Universitätsklinikum Leipzig (Germany)

Onychomycosis is a worldwide problem with no tendency for self-healing and available systemic treatment achieve a disease-free nail in only 35-76 %. Recently, the option of near infrared laser nail fungus treatment has been introduced. It is assumed that the eradication effect is mediated by heat. Therefore laser heat application and propagation needs to be studied to answer the question if the proposed lasers treatment regimens do have the potential to eradicate fungi and spores.

To address these questions this study aimed on measuring nail temperatures by real time videothermography during laser irradiation to (1) estimate peak temperatures, (2) to establish average temperatures profiles, and (3) to analyze the heat propagation. Laser treatment was performed using 808 and 980-nm linear scanning diode laser introduced for hair removal enabling a contact free homogeneous irradiation of a human nail plate in one pass.

In general average and peak temperatures measured during the laser beam movement along the nail plates increased pass by pass. Peak temperatures (808 nm: 74.1 °C to 112.4 °C, 980 nm: 45.8 °C to 53.5 °C) as well as average temperatures (808 nm: 29.5 °C to 38.2 °C, 980 nm: 27.1 °C to 32.6 °C) related to a pain which was equivalent to hair removal procedures and not significantly different between the wavelengths.

Real time video thermography recording of a laser intervention therefore allow to set up average and peak temperature profiles in human nails for a given laser system. Linear scanned laser devices previously used for hair removal do inherit the advantage of a contact free homogeneous heating of the human nail by ensuring adequate raise in temperatures.

8926-11, Session 3

Latest advances in confocal microscopy of skin cancers: early signs of impact on patient care *(Invited Paper)*

Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Reflectance confocal microscopy (RCM) is a noninvasive approach for imaging skin cancer, with optical sectioning and resolution that shows nuclear, cellular and architectural morphology. Basal cell carcinoma (BCCs) can be detected in vivo with sensitivity of 92-100% and specificity of 97-85%, and melanocytic lesions with 92-88% and 70-84%. The specificity is twice that of dermoscopy, especially for non-pigmented lesions. Lentigo maligna melanoma can be detected with sensitivity of 93% and specificity 82%. The existing studies report Level II-evidence that RCM could reduce the number of biopsies of benign lesions by up to 68%. Imaging, mainly near the dermo-epidermal junction, is being shown to reduce the need for biopsy for diagnosing benign lesions. Pre- and intra-operative imaging is being implemented for guiding treatment choices for LMMs and management of patient care. With fluorescence confocal mosaicing microscopy, residual BCC can be detected in Mohs surgically excised fresh tissue ex vivo, with sensitivity of 94-97% and specificity 89-94%. The mosaicing approach was demonstrated at the bedside, for rapid examination of shave biopsies to guide treatment choices and of surgical excisions to guide Mohs surgery. These latest advances, during 2013, demonstrate early signs of impact on patient care.

8926-12, Session 3

Computer based algorithm for estimating stratum corneum thickness from reflectance confocal microscopy (RCM) images

Alican Bozkurt, Bilkent Univ. (Turkey); Kivanc Kose, Memorial Sloan-Kettering Cancer Ctr. (United States); Jamshid Sourati, Northeastern Univ. (United States); Christi Alessi-Fox, Caliber Imaging & Diagnostics, Inc. (United States); Jennifer G. Dy, Dana H. Brooks, Northeastern Univ. (United States); Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Thickness estimation of the stratum corneum (SC) is often required in pharmacologic, dermatologic and cosmetologic work. Reflectance confocal microscopy (RCM) is a non-invasive technique for high-resolution imaging of cellular layers in skin. Although automated SC thickness estimation algorithms are available for other imaging modalities such as optical coherence tomography, multi-photon microscopy, and Raman-spectroscopy, RCM based SC measurements currently rely on visual analysis of images, which is subjective and variable. Here, we propose an algorithm to automatically estimate the thickness of SC in RCM stacks. Most RCM image stacks exhibit artifacts caused by

reflections from the objective lens-to-skin contact window routinely used with the microscope. To reduce these artifacts and the noise, we first preprocess the data. Each image in the stack is then divided into small regions ("tiles"). Image features, specifically the number of non-zero elements of the gray-level co-occurrence matrices calculated for 4 angles (0°,45°,90°,135°) and 4 steps (1,2,3,4) neighbors, are extracted from each tile. We cluster features into 2 classes, using k-means clustering (a standard unsupervised machine learning algorithm) to avoid having to manually label data to train the algorithm, thus reducing labor and increasing robustness across different skin types. Each tile of each slice is classified, using its features, to create a classification mask. The thickness is calculated from the mask, after a post-processing stage in order to obtain smooth clusters,. When tested on 15 RCM stacks of skin acquired in vivo, our algorithm measured the thickness of the stratum corneum with an accuracy error of ~5.25um.

8926-13, Session 3

Image analysis based automated detection of dermal-epidermal junction in reflectance confocal microscopy images of skin

Kivanc Kose, Memorial Sloan-Kettering Cancer Ctr. (United States); Christi Alessi-Fox, Caliber Imaging & Diagnostics, Inc. (United States); Jamshid Sourati, Jennifer G. Dy, Dana H. Brooks, Northeastern Univ. (United States); Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Clinical and pathological examination of the dermal-epidermal junction (DEJ) along with the cellular and morphological structures in its vicinity is important for skin cancer diagnosis, since most skin cancers typically originate at and spread from the DEJ. However, visual delineation of the DEJ with reflectance confocal microscopy (RCM) is subjective and requires significant reader training due to the en face orientation of images, loss of resolution, loss of contrast (especially in lightly pigmented skin) and speckle noise. Therefore, automated DEJ detection is of great interest. We previously reported a machine learning based algorithm for automated DEJ localization based on textural features. However, due to high variability in skin data (heterogeneity of skin), this method was difficult to generalize to larger datasets. In this study, we report a more robust and computationally efficient algorithm. We first denoise the images using an edge-adaptive filter, and then enhance contrast by non-linearly stretching the image intensity scale. Then we analyze the RCM stack tile by tile, to locate the depth range in which there is decrease in the resolution (blurring) due to crossing the DEJ. We then further analyze this region using an intensity histogram-based peak detection method to locate basal layer and the DEJ. The proposed algorithm effectively cleans noise, enhances the contrast, and detects the location of the DEJ with a median error of ~7.5um in 16 pigmented skin stacks and ~35um in 5 lightly pigmented skin stacks. Further improvement can be achieved by combining the method with machine learning based analysis.

8926-14, Session 3

Feasibility of intraoperative imaging during Mohs surgery with reflectance confocal microscopy

Eileen S. Flores, Miguel Cordova, William Phillips, Kishwer S. Nehal, Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Mohs surgery for the precise microexcision of basal cell cancers (BCCs) is performed in stages, while being guided by the examination for residual tumor with frozen pathology. However, preparation of frozen pathology at each stage is time-consuming and laborious. Real-time intraoperative reflectance confocal microscopy (RCM) may enable rapid detection of residual BCCs directly in surgical wounds on patients. We report initial feasibility on twenty patients, using 35% AlCl3 for nuclear contrast. Imaging was performed in quadrants in the wound, to simulate the Mohs surgeon's examination of pathology. Images and videos of the epidermal and dermal margins were found to be of clinically acceptable quality. Bright nuclear morphology was identified at the epidermal margin. The presence of residual BCC tumor and normal skin features could be detected in the peripheral and deep dermal margins. Nuclear morphology was detectable in residual BCC tumors. Intraoperative RCM imaging may enable detection of residual tumor, directly on Mohs patients, and may serve as an adjunct for frozen pathology. However, a stronger source of contrast will be necessary, and also a smaller device with an automated approach for imaging in the entire wound in a rapid and controlled manner for clinical utility.

8926-15, Session 3

Laser ablation of basal cell carcinoma guided by confocal microscopy: effect of fluence, number of passes, contrast agent and residual thermal damage on imaging quality

Heidy Sierra, Jason Chih-Shan Jason Chen, Milind Rajadhyaksha, Memorial Sloan-Kettering Cancer Ctr. (United States)

Laser ablation is a less invasive alternative to standard Mohs surgery and surgical excision for the removal of superficial basal cell carcinomas (BCCs). Advantages of laser ablation include finely controlled removal of tissue, faster recovery and better cosmetic outcomes. However, tissue is vaporized and not thus available for subsequent histopathological examination for residual BCCs. Reflectance confocal microscopy (RCM) may offer an imaging approach for detection of residual BCCs directly on the patient, and may guide ablation. Last year, we reported preliminary feasibility with an Er:YAG laser at wavelength of 2940nm. This year, we will present the results of further experiments in skin tissue ex-vivo, to determine the optimum parameters (fluence, number of passes) for ablation of BCC with controlled residual thermal damage to allow subsequent RCM imaging of residual tumor. Contrast agents (acetic acid or aluminum chloride) were tested for enhancing the appearance of nuclear morphology and BCCs. We studied the effect of residual thermal damage and char formation on imaging quality. The results show that, after ablating deeper than ~150 μm of tissue, nuclear morphology can be detected and residual BCC distinguished from normal skin in RCM images. Through char of thickness ~30μm, epidermis and dermis are well visualized in ablated tissue, and observed morphology correlates well to that seen in histopathology. Thus, an optimal protocol for ablation, with control of residual thermal damage and char, may allow RCM imaging to guide ablation and improve the efficacy and reliability of this treatment approach.

8926-16, Session 4

Integrated multiphoton microscopy and micro-Raman spectroscopy system for accurate targeting and biochemical analysis of microstructures in human skin In vivo

Hequn Wang, Anthony M. Lee, The BC Cancer Agency Research Ctr. (Canada); Harvey Lui M.D., David I. McLean M.D., The Univ. of British Columbia (Canada); Haishan Zeng, The BC Cancer Agency Research Ctr. (Canada) and Univ. of British Columbia (Canada)

Acquiring confocal Raman spectrum of in vivo skin tissues takes tens of seconds. The movement from the subjects could change the

measurement volume, leading to non-specific signals. This will undermine the interpretation accuracy of the acquired spectrum. Our objectives are to develop an integrated system combining reflectance confocal microscopy (RCM), multiphoton microscopy (MPM), and micro-Raman spectroscopy and to introduce a method to achieve accurate spectral measurement and precise biochemical interpretation of the spectra. This method includes (1) developing a multimodal system to achieve better identification of interesting microstructures and real-time monitoring of every spectral measurement with RCM imaging and MPM imaging; (2) performing region-of-interest measurement by scanning the targeting tissue microstructure during spectral acquisition. The developed system and method have been validated by measuring different microstructures of in vivo human skin. Our results demonstrated great consistency between RCM/MPM images and confocal Raman spectra. The superior quality of the images and spectra allows us to derive blood flow velocity and blood glucose level. We believe that this new method is valuable for realizing accurate microscopic spectral measurement/biochemical analysis and have great potential to be adapted into skin clinic to achieve non-invasive measurement of a variety of important biological parameters.

8926-17, Session 4

Sensing vascularization of ex vivo produced oral mucosal equivalnent (EVPOME) skin grafts in nude mice using optical spectroscopy

Karthik Vishwanath, Rajan Gurjar, Radiation Monitoring Devices, Inc. (United States); Shiuhyang Kuo, Anthony Fasi, Roderick Kim, Stephen E. Feinberg, Univ. of Michigan (United States); David E. Wolf, Radiation Monitoring Devices, Inc. (United States)

Repair of soft tissue defects of the lips as seen in complex maxillofacial injuries, requires pre-vascularized multi-tissue composite grafts. Protocols for fabrication of human ex-vivo produced oral mucosal equivalents (EVPOME) composed of epithelial cells and a dermal equivalent are available to create prelaminated flaps for grafting in patients. However, in-vivo assessment of vascularization of the buried prelaminated flaps remains clinically challenging.

Here, we use diffuse reflectance spectroscopy (DRS) and diffuse correlation spectroscopy (DCS) to non-invasively quantify longitudinal changes in the vessel density and blood-flow within EVPOME grafts implanted in the backs of SCID mice and subsequently to determine the utility of these optical techniques for assessing vascularization of implanted grafts.

20 animals were implanted with EVPOME grafts (1x1x0.05 cm3) in their backs. DRS and DCS measurements were obtained from each animal both atop the graft site and far away from the graft site, at one week post-implantation, each week, for four consecutive weeks. DRS spectra were analyzed using an inverse Monte Carlo model to extract tissue absorption and scattering coefficients, which were then used to extract blood flow information by fitting the experimental DCS traces.

There were clear differences in the mean optical parameters (averaged across all mice) at the graft site vs. the off-site measurements. Both the total hemoglobin concentration (from DRS) and the relative blood flow (from DCS) peaked at week 2 at the graft site and declined to the off-site values by week 4. The optical parameters remained relatively constant throughout 4 weeks for the off-site measurements.

8926-18, Session 4

Comparison of cone and cone shell configuration for depth sensitive fluorescence measurements in turbid media

Yi Hong Ong, Quan Liu, Nanyang Technological Univ. (Singapore)

Ultraviolet-visible fluorescence spectroscopy has been explored in the detection of precancers in human epithelial tissues as vital diagnostic information regarding tissue morphological and biochemical changes can be extracted from the endogenous fluorophores. The depth distribution of these endogenous fluorophores is affected by age, menopausal status (for women), and disease state. Therefore, a depth sensitive probe that can measure fluorescence spectra as a function of depth will enhance the diagnostic performance of this technique. Generally, a lens based setup uses a single lens or a combination of lenses to achieve a cone configuration, in which both the excitation and emission volumes in an optically transparent medium would form light cones. One weakness of this setup is the limited sensitivity to subsurface fluorescence due to the dominance of fluorescence from shallower layers. Furthermore, it would be required to vary the distance between the imaging lens and the tissue sample in order to measure fluorescence from different depths. This would induce uncertainty in optical coupling and cause great inconvenience in clinical measurements.

In this study, we have developed a novel non-contact setup to implement a cone shell illumination and detection configuration using multiple axicon lenses for depth sensitive fluorescence measurements. The setup was demonstrated experimentally capable of detecting fluorescence from a two-layered turbid agar phantom with a larger sensitivity to the deep layer and a larger range of sensitivity to either layer than a conventional cone configuration implemented by a microscope objective lens. Furthermore, the axicon lens based setup eliminates the need of altering lens-sample distance to achieve depth sensitive measurements, which effectively improves the consistency of optical coupling thus would be preferred in a clinical setting.

8926-19, Session 4

Using parallel factor analysis (PARAFAC) for decomposing excitation and emission matrix (EEM) spectra of healthy skin according to body site

Wenbo Wang, Jianhua Zhao, Haishan Zeng, The BC Cancer Agency Research Ctr. (Canada); Harvey Lui M.D., The Univ. of British Columbia (Canada)

Excitation-emission matrix (EEM) fluorescence spectroscopy measures fluorescence spectra at multiple excitation wavelengths. The resulting matrices can be used to identify sample fluorophores and their concentrations. In skin tissue, several important endogenous fluorophores such as tryptophan and nicotinamide adenine dinucleotide (NADH) exhibits fluorescence features that appear as peaks in the EEM spectra. An efficient method to separate excitation and emission information in resolving EEM spectra of complicated samples such as human skin is considered highly desirable. The underlying chemical phenomena of fluorescence enable multi-way modeling methods, e.g., Parallel Factor Analysis (PARAFAC) in particular, to be used as suitable tools for exploratory analysis of EEM spectra. In this study, PARAFAC was applied to analyze EEM spectra of human skin measured at different body sites. A group of 30 healthy patients were recruited and EEM measurements were taken at 10 different body sites. By varying the number of factors, different PARAFAC models were trained to model the underlying structures of skin EEM spectra. Excitation and emission wavelength pairs were extracted and compared favorably with hand-picked peak values in EEM spectra. It was found that a model factor number of 3 provided adequate model complexity for most body site

measurements. The optimal number of factors was determined based on core consistency diagnostic values and other parameters. The advantages of PARAFAC to be able to extract pure fluorescence spectra and relative concentrations of individual fluorophores make it a powerful tool in elucidating the histological states of human tissue.

8926-20, Session Key

Optical treatment strategies for unsolved skin problems: pros, cons, and some whacky ideas *(Keynote Presentation)*

R. Rox Anderson M.D., Massachusetts General Hospital (United States)

The optical toolbox for skin treatment includes UV phototherapy, light-activated drugs, surgical lasers, flashlamps, optical nanoparticles and so-called low level light sources. We also have some remarkably interesting in vivo microscopes and optical diagnostics. These and others were motivated for treatment of a handful of the appropriately 2000 skin diseases. A few have provided miracle cures, many are useful but could use major improvement, and some just don't work well enough to be useful. Taking a problem-driven approach, in view of known mechanisms and capabilities in our optical toolbox - where might we make some strides?

8926-21, Session 5

New ways to deliver PDT with daylight and fractional lasers: emerging techniques and protocols *(Invited Paper)*

Merete Haedersdal M.D., Bispebjerg Hospital (Denmark)

Topical PDT is a mainstream treatment for premalignant lesions and selected cases of non-melanoma skin cancer (NMSC). In Europe, PDT traditionally is delivered with methylaminolevulinic acid (MAL) and red LED light, which is highly effective for thin dysplastic lesions. However, PDT is time consuming, painful, and less effective for thick lesions. There is, therefore, a need to look for alternative ways of delivering PDT.

New emerging techniques include daylight-mediated PDT (day-PDT) and ablative fractional laser-assisted PDT (AFXL-PDT). Day-PDT is a low-pain, easy-to-do procedure, which is highly effective for particularly thin actinic keratosis, and part of the procedure being performed in a home-based setting. AFXL-PDT is a new intensified treatment concept with promising data for the treatment of thick lesions in both immunocompetent and immunosuppressed, organ transplant patients. Accumulating evidence is obtained for both day-PDT and AFXL-PDT.

This presentation will give up-to-date information on the current status to use day-PDT and enhanced AFXL-PDT for NMSC and precursor lesions. Data will be presented from basics to clinics, focusing on in vivo experimental lab data and new clinical studies; thus translating new treatment protocols into clinical benefit for patients with dysplastic skin lesions. It is suggested to take advantage of these new treatment advances to individualize the way of delivering PDT for different types of patients

8926-22, Session 5

Photodynamic therapy improves the ultraviolet-irradiated hairless mice skin

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Chronic exposure to ultraviolet (UV) sunlight causes premature aging skin. In light of this fact, photodynamic therapy (PDT) is an emerging modality for treating cancer and other skin conditions, however its response on photoaged skin has not been fully illustrated by means of histopathology. For this reason, the aim of this study was analyze whether PDT can play a role on a mouse model of photoaging. Hence, SKH-1 hairless mice were randomly allocated in different groups, such as Control, UV, and UV/PDT. The mice were daily exposed to an UV light source (280-400 nm: peak at 350 nm) for 8 weeks followed by a single PDT session using 20% 5-aminolevulinic acid (ALA) topically. After the proper photosensitizer accumulation within the tissue, a non-coherent red (630 nm) light was performed and, after 2 weeks apart, skin samples were collected and stained by hematoxylin-eosin (HE) and Masson's Trichrome. As a result, we observed a substantial epidermal thickening - up to 5 fold - and an improvement in collagen density by deposition of new collagen fibers on UV/PDT group. These findings strongly indicate epidermal and dermal restoration, and consequently skin recovery. In conclusion, this study provides suitable evidences that PDT improves the UV-irradiated hairless mice skin, supporting this technique as an efficiently treatment for photoaged skin.

8926-23, Session 5

Preclinical in-vivo evaluation of Hemoporfin-mediated photodynamic therapy on normal vasculature

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Port wine stain (PWS) is a congenital birthmark commonly found on the face and neck regions. It consists of abnormally-enlarged vasculature in the dermis. To treat PWS, clinicians use optical methods to achieve selective photocoagulation of the targeted vasculature. Typical treatment protocols involve the use of a pulsed-dye laser (PDL) combined with cryogenic cooling of the skin. In China, researchers are evaluating photodynamic therapy (PDT), primarily with the photosensitizer Hemoporfin.

We have characterized PDT protocols involving NPe6 and BPD as photosensitizers. In this study, we compared the effectiveness of Hemoporfin-mediated PDT to our previously characterized photosensitizers in the same preclinical model. We hypothesize that Hemoporfin-mediated PDT achieves vascular shutdown with similar efficacy to NPe6 and BPD.

We utilized a previous protocol involving use of the mouse dorsal window chamber model and laser speckle imaging to monitor longitudinal blood-flow dynamics. We defined a successful treatment outcome as achieving persistent vascular shutdown within the window, seven days following the PDT treatment.

With our preclinical data, we applied dose-response curve analysis to identify a characteristic radiant exposure required to achieve a successful treatment. We also studied irradiance-dependent effects on the efficacy of Hemoporfin-mediated PDT. Our preliminary data suggests that the Hemoporfin-mediated PDT is a viable treatment option for PWS vasculature.

8926-24, Session 6

High resolution in-vivo imaging of skin with full field optical coherence tomography

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Full-field OCT (FFOCT) has the ability to provide en-face images with a very good axial sectioning as well as a very high transverse resolution (about 1 microns in all directions). Therefore it offers the possibility to visualize biological tissues with very high resolution both on the axial native view, and on vertical reconstructed sections. Here we investigated the potential dermatological applications of in-vivo skin imaging with FFOCT.

A commercial FFOCT device was adapted for the in-vivo acquisition of stacks of images on the arm, hand and finger. Several subjects of different benign and pathological skin conditions were tested. The images allowed measurement of the fingerprints, measurement of the stratum corneum and epidermis thicknesses, size measurement and count of the keratinocytes, visualization of the dermal-epidermal junction, and visualization of the melanin granules and of the melanocytes. Skins with different pigmentations could be discriminated and skin pathologies such as eczema could be identified.

The very high resolution offered by FFOCT both on axial native images and vertical reconstructed sections allows for the visualization and measurement of a set of parameters useful for cosmetology and dermatology. In particular, FFOCT is a potential tool for the understanding and monitoring of skin hydration and pigmentation, as well as skin inflammation.

8926-25, Session 6

Noninvasive monitoring and differentiation of cell death processes of human keratinocytes in living engineered skin tissue

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Apoptosis and necrosis are the two major pathways of cell death. Detection and imaging of apoptosis and necrosis in vivo is not only important for investigating development and homeostasis under normal physiological conditions, but also crucial for monitoring therapeutic efficacy, particularly in cancer treatment. While apoptosis and necrosis have been well defined histologically, noninvasive imaging offering subcellular resolution, molecular specificity, and the capability of distinguishing and monitoring different cell death processes in vivo is needed for both clinical practices and basic researches. In this work, we exploit real-time tracking of apoptotic and necrotic cells in living tissues using a multimodal microscope. The microscope combines the functions of optical coherence (OCM) and multiphoton (MPM) microscopy providing three-dimensional (3-D) co-registered time-lapse images that carry comprehensive information including structural light scattering, molecular autofluorescence, and metabolic changes. Cell death dynamics (apoptosis, necrosis, cornification) of keratinocytes were investigated longitudinally in living engineered human skin, which was found much more complex than the cultured cells used in recent studies of cell death with single optical imaging techniques. Despite the limited capabilities of individual modalities, we show that high confidence discrimination of different cell death pathways is attainable based on the multidimensional data provided by the integrated microscope. Differentiation of the three cell death processes is enabled by quantitative analysis based on effective cell segmentation and classification algorithms using the mutually validating, multidimensional data, which cannot be obtained with any single modality. This non-invasive, label-free multimodal imaging approach has the potential for in vivo studies of cell death processes and real-time clinical monitoring.

8926-26, Session 6

Clinical CARS tomography

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Coherent anti Stokes Raman scattering (CARS) microscopy enables highly sensitive, label-free imaging thus providing the possibility of three dimensional imaging of tissue and skin. We present measurement results of combined epi CARS and multiphoton microscopy, hence, label-free imaging of pig skin ex vivo with both chemical discrimination and subcellular resolution. In particular, we show how epi-CARS microscopy is utilized to image lipid-rich structures inside the skin, preparing for a combined multiphoton and CARS imaging modality for biomedical research and skin imaging.

8926-27, Session 7

Estimation of skin optical parameters for real-time hyperspectral imaging applications

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Hyperspectral imaging can be used to analyze the chemical composition of tissue using spectroscopic methods. This can be applied as a general purpose real-time diagnostic tool.

Light transport models, like the diffusion model, can describe light propagation in tissue. The aim of this project is to create an inverse light transport model to extract optical parameters from hyperspectral images of skin. This should be done in real-time, i.e. within a deadline defined by the speed of the hyperspectral camera, which is 30 ms per line of data in our case.

A two-layered skin model is used to estimate the melanin content in epidermis and the dermal absorption coefficient. The skin properties are fitted to the absorption coefficients using a non-negative least squares algorithm at separate wavelength intervals. The approach is implemented in CUDA for parallelization on Graphics Processing Units.

The resulting inversion chain was found to meet the deadline, finishing the results after 3.5 ms for 1600 pixels, 160 bands and three wavelength intervals. The inversion approach was found to characterize the relative variations of the optical parameters in hyperspectral images of wounds and normal tissue and the absolute values were found to be within physical levels. The inversion routine was tested using Monte Carlo simulations and found to characterize the relative variations in the parameters.

The developed hyperspectral inversion module can be used to estimate skin parameters in real-time. This will be one of many necessary processing blocks in a future real-time diagnostic system using hyperspectral imaging.

8926-28, Session 7

Quantitative fluorescence molecular imaging in highly light-absorbing melanomas using a dual-tracer kinetic modeling normalization method

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Tissues with high light absorption, such as melanomas, present a significant challenge to fluorescence imaging approaches that seek to estimate molecular expression in vivo, since any fluorescence originating in the tissue will suffer substantial attenuation prior to detection. This can lead to sizable underestimations in estimated fluorescent tracer concentration in these tissues using conventional fluorescence imaging. In this study, a dual-tracer fluorescence imaging approach was employed to correct for severe tissue absorption by 1) using simultaneous injection and imaging of an untargeted tracer to normalize tissue absorption effects on the targeted tracer, and 2) using kinetic modeling that capitalizes on subtle differences in the dynamics of targeted and untargeted tracer uptake to quantify targeted molecule concentrations in the high absorbing tissue. Monte Carlo simulation and kinetic models demonstrated that the effect of optical properties on the approach could be eliminated by a pixel-by-pixel normalization of the targeted and untargeted tracer uptakes prior to 5 min post-tracer injection for fluorescence planar dynamic imaging. Moreover, in an in vivo study looking at PV1 expression (an intravascular marker exposed in tumor vascular) in transgenic mice with induced melanomas, it was shown that a significant overexpression of PV1 could be identified in the melanomas compared to healthy skin through dual-tracer imaging, despite an order of magnitude lower fluorescence measured in the melanomas.

8926-29, Session 7

Combining the diffusion approximation and Monte Carlo modeling in analysis of diffuse reflectance spectra from human skin

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Measurement of diffuse reflectance spectra (DRS) is a popular experimental technique for non-invasive determination of tissue optical properties, as well as objective characterization of various tissue malformations. In the latter attempts, propagation of light in strongly scattering biological tissue is often treated within the diffusion approximation (DA). The major advantage of DA is that it leads to enclosed analytical solutions for diffuse reflectance from tissues with layered structure, such as human skin. Despite the fact that the DA solutions are known to be inaccurate near tissue boundaries and absorbing layers [1,2], the practicality of this approach makes it quite popular, especially when it comes to solving the inverse problem of extracting skin lesion characteristics from DRS (e.g. concentrations of epidermal melanin and dermal blood, oxygen saturation level, etc.)

In our study, we will analyze the artifacts in skin composition parameters, arising when fitting the DRS with the DA solutions for two- and three-layer skin models. We will then demonstrate an original procedure, which enables us to markedly improve the accuracy of extracted skin composition parameters. The correction is based on two comparison runs of the more accurate Monte Carlo (MC) model, but avoids the need to implement and run an inverse MC. The performance of our approach will be tested in analysis of seasonal changes of DRS in healthy human skin.

[1] T. Spott and L. O. Svaasand, Appl. Opt., vol. 39, 6453–6465 (2000)

[2] L. L. Randeberg et al., Proc. SPIE, vol. 5862, 58620O (2005).

8926-30, Session 7

Monte Carlo modeling of pigmented skin lesions

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Colors observed in clinical dermoscopy are critical to diagnosis but the mechanisms that lead to the spectral components of diffuse reflectance are more than meets the eye: combinations of the absorption and scattering spectra of the biomolecules involved as well as the “structural color” effect of skin anatomy. We report on modeling of diffuse light remittance from skin microstructures. Pathology was processed to derive spatial models of skin consisting of 4 tissue types: stratum corneum, superficial epidermis (granular and spinous layers), basal epidermis and dermis. The optical properties of these tissue types were modeled based on the content of absorbers and scatterers expected to be present: compressed scattering keratin in the stratum corneum, keratin and low-concentration melanin in the superficial epidermis, extra melanin in the basal layer and melanocytic nests as well as blood in the dermis. The results of the simulation are spectral images (such as Red/Green/Blue) that mimic the appearance of pigmented lesions quite well when the morphology is mathematically derived but limited when based on histopathology, raising interesting questions about the mechanism of structural colors in skin.

8926-31, Session 7

In vivo validation of a method to isolate the effects of melanin from underlying hemodynamics across skin types using spatially modulated quantitative spectroscopy (SMoQS)

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Skin is a highly structured and layered tissue. This presents unique challenges to quantitative spectroscopic techniques that rely on homogeneous models. In order to more accurately address the challenges associated with skin, we have developed a method that enables depth-resolved quantification of in-vivo skin optical properties and chromophore concentrations using on a two layer model. While this empirical method has demonstrated success in separating and quantifying layer-specific chromphore concentrations in simulation and structured tissue phantom studies, its application across a wide range of skin types has yet to be validated.

We present a study employing 15 subjects ranging in skin type II through VI (Fitzpatrick scale). A blood pressure arm cuff has been employed in order to validate the separability of melanin from a controlled perturbation in hemodynamics. Data has been acquired using SMoQS and analyzed

via our two layer approach. High frequency ultrasound was also collected to independently confirm epidermal thickness which another measure determined through this approach. Results suggest that there is a significant reduction in the amount of cross-talk between melanin and hemoglobin concentration changes using the layered model relative to that determined by standard spectral decomposition methods.

8926-32, Session 7

Enhanced diagnostic of skin conditions by polarized laser speckles: phantom studies and computer modeling

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The incidence of melanoma of the skin, the most commonly fatal form of skin cancer, is increasing faster than any other potentially preventable cancer. The World Health Organisation proposes that early detection of cancer is crucial in cancer management in primary care settings. Clinical practice is currently hampered by the lack of the ability to rapidly screen the functional and morphological properties of tissues at depth. We explore the use of laser image speckle approach to map spatial distribution of coherence and state of polarization of scattered laser light over the entire lesion surface of human skin, in order to differentiate the most aggressive form of skin cancer such as malignant melanoma. In this study we exploit the phantoms of skin with variable roughness and bulk optical properties. The results of experimental measurements are compared with the results of Monte Carlo simulation. In the developed Monte Carlo model, polarization, coherence of laser light and roughness of the medium surface are taken into account. We demonstrate that image intensity and polarization speckle allow to differentiate phantoms with different optical properties better than free-space speckle.

8926-33, Session PSun

Fractal feature of basal cell carcinoma caused by excessive ultraviolet radiation

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Fractal geometry is a tool to characterize irregularly shaped and complex figures. It can be used not only to generate biological structures, but also to derive the fractal dimension in order to quantify the shapes of structures. In our study, the images during the process of basal cell carcinoma (BCC) irradiated by excessive ultraviolet radiation in the hairless mouse were monitored by skin detector in vivo. A box-counting algorithm was applied to determine the fractal dimension in order to evaluate the BCC damage. Based on histological analysis, the relation between morphology and fractal feature was established. The results help us to understand the mechanism of BCC from texture feature.

8926-34, Session PSun

Development of a widefield imaging fluorescence system for detection of porphyrins in dermatology

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Acne is the most common skin disease found by dermatologists, where it is considered chronic because it has a high degree of recurrence may affect 50% of the population in adult life. Several studies have shown good results for treatment of acne via phototherapy, however, some studies emphasize that this type of treatment is still new and additional and more detailed studies should be conducted. This paper presents the development and validation of equipment, based on fluorescence images that monitor the protoporphyrin IX (PpIX), is for use as diagnosis of acne-causing bacteria, such as its use in monitoring formation, accumulation and photobleaching of PpIX during PDT applied to acne. The equipment developed consists of an optical system, mechanical, electronic and detection. The optical part consists of a high intensity LED (405 nm) and 5 optical components: 1 - bandpass filter, 2 - notch filter, 3 - longpass filter, 4 - dichroic beamsplitter, 5 - anti-reflection filter and a biconvex lens. Four distinct experiments were performed in order to validate the detection of bacteria present on the face or scalp of volunteers. It is too early a conclusive analysis of the efficiency of detection of fungi and bacteria and its correlation with the cause of dermatitis and acne, however, initial tests point to this direction. The prototype shows good intensity and uniformity of illumination and good quality images for the detection of fluorescence in the red area, provided by the choice and optimization of optical filters used.

8926-35, Session PSun

Microneedles rollers as a potential device to increase ALA diffusion and PpIX production: evaluations by wide-field fluorescence imaging and fluorescence spectroscopy

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One of the limitations of topical photodynamic therapy (PDT) using 5-aminolevulinic acid (ALA) is the poor ability to penetrate biological barriers of skin and the recurrence rates in treatments. This study aimed to identify possible signs of increased diffusion of ALA-induced PpIX by fluorescence images and fluorescence spectroscopy. The research was done using in vivo porcine skin model. Before the cream application, microholes was performed with microneedles rollers in only one direction, afterward the ALA cream was applied at a 2.5cm2 area in triplicate and an occlusive dressing was placed. PpIX production was monitored using fluorescence spectroscopy collected at skin surface after 70, 100, 140, and 180 minutes of ALA incubation. About 100 fluorescence spectra of each treatment were collected, distributed by about five points for each site. Wide-field fluorescence imaging was made after 70, 90, and 170 minutes after treatment. The results obtained by imaging analysis indicated increase of the PpIX diffusion in the skin surface using the microneedles rollers (MNs) before ALA application. Circular regions of red fluorescence around the microholes were observed. In addition, the fluorescence spectra showed a greater intensity (2 times as many) in groups microneedles rollers associated. In conclusion, our data shown greater homogeneity and PpIX production in the groups pre-treated with microneedles indicating that the technique can be used to greater uniformity of PpIX production throughout the area to be treated reducing the chances of recurrent tumor as well as has potential for decreasing the time of therapy. (FUNDING SUPPORT:CAPES, CNPq and FAPESP)

8926-36, Session PSun

Raman spectroscopy could measure the differences in the biochemical constitution of human skin in different regions of the body

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Human skin is an important organ, protecting against chemical, physycal and biological aggressors, and being important in the body's thermal control. Its physical characteristics and biochemical constitution may present differences depending on the site of the body, race and skin phototype. The characterization of the skin constitution and its differences are of interest of the medicine and recently of the cosmeceutics. The use of vibrational techniques such as Raman spectroscopy could help identify the differences in the biochemical constitution of the skin, in vivo. This work measured the Raman spectra of normal skin in vivo, and a spectral model based on least squares fitting was developed to estimate the relative concentration of selected biochemicals, measured in four different sites: hand, forearm, forehead and face. It has been used a dispersive Raman spectrometer (830nm, 200mW) connected to a Raman probe. Results indicated that there are differences in the relative concentration of the selected biochemicals depending on the site of the skin: hemoglobin and elastin were increased from hand to forearm, elastin was decreased from forearm to face, triolein was increased from hand and forearm to forehead and face. These differences could be attributes to the differences in the skin thickness and constitution depending on the site. The deviation could be explained by the fact that different phototypes were included in the model and also the higher penetration depth of the laser used (about 4.7 mm), that illuminates the deeper layer (dermis and muscular layer).

8926-37, Session PSun

Using infrared imaging polarization imaging to detect skin cancer

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We are developing an imaging technique to detect skin cancer using polarization measurements at thermal infrared (9-10 microns) wavelengths. Skin cancer often develops in the superficial layers of the skin. Thermal emission originates in these superficial skin layers and polarization measurements of this emission can characterize the properties of these layers. Previous investigators have demonstrated that optical polarized light imaging of the superficial layers of skin defined the borders of skin cancer that were not clearly visible to the naked eye (Ramella-Roman et al. 2004). Our technique does not require the outside light source or preferred reflection geometry needed for the optical techniques. We have measured the degree of linear polarization of thermal emission from porcine skin samples held at body temperature in a saline bath. We used a thermal camera (25x18.8 degrees and .08 mm per pixel) and a wire grid polarizer to acquire polarization maps of the tissue samples. We then used an IR laser to create visible thermal lesions (2 mm) on the tissue samples. We then acquired polarization imaging of the tissue samples after thermal damage. The measured degree of linear polarization was 5-8% and was approximately uniform across each skin sample. After thermal damage, a clearly defined area of lower polarization (drop of a 2-3%) corresponding to the thermal lesion was evident on all tissue samples. These preliminary results indicate that our imaging technique could be used to detect abnormalities in tissue structures associated with the development of skin cancer lesions.

8926-38, Session 8

Photothermal laser speckle imaging

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When coherent light shines on a rough or dispersive object, the scattered light forms a random interference pattern called speckle. If the object remains at rest, the speckle pattern will remain static; if the object is moving the speckle pattern changes over time and is called dynamic speckle. When the fluctuating speckle pattern is integrated over time for a CCD camera, the speckle pattern gets blurred, resulting in a decrease of the contrast of the image. Based on this idea, Fercher and Briers [1] demonstrated that analysis of the speckle contrast in a time-integrated speckle pattern enables visualization of superficial blood flow in exposed vasculature, a method we call Laser Speckle Imaging (LSI). With current methods, LSI does not enable visualization of subsurface or small vasculature, due to optical scattering by stationary structures. In this work we propose a new technique called Photothermal-LSI to improve visualization of blood vessels. A 595nm laser pulse was used to excite a blood-filled, microchannel-based skin phantom. The high absorption coefficient of blood at this wavelength results in efficient conversion of the optical energy to heat energy, resulting in an increase in the local temperature and hence increased scatterer motion, resulting in a transient decrease in speckle contrast. As a result we found that Photothermal-LSI was able to visualize blood vessels that were hidden for a standard LSI system.

[1] A. Fercher and J. Briers, "Flow visualization by means of single-exposure speckle photography", Opt. Commun. 37, 326-330 (1981).

8926-39, Session 8

Tumor site prediction using spatiotemporal detection of subclinical hyperemia in experimental photocarcinogenesis

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We demonstrate that a spatial and temporal analysis of subclinical hyperemia reliably predicts specific areas at high risk for skin tumor development during photocarcinogenesis. To determine detailed spatiotemporal patterns of inflammatory angiogenesis foci in a relatively large area, we developed a mesoscopic (between microscopic and macroscopic) imaging approach. This method relies on our earlier finding that the combination of a spectral analysis of hemoglobin (Hgb) with diffuse-light-suppressed imaging can increase the image resolution, contrast and penetration depth to visualize microvasculature Hgb content in the large tissue area. In this study, SKH1 hairless albino mice were irradiated for 10 weeks with a carcinogen dose of UVB. After stopping UVB, we imaged the mice over 20 - 30 weeks and excised hyperemic/non-hyperemic areas at several different time-points. We show that persistent hyperemic foci can predict future tumor formation. In particular, our imaging approach allows us to assess the spatial and temporal extent of subclinical inflammatory foci, which in turn can predict sites of future overlying tumor formation. In addition, although COX-2 inhibitors are known to suppress skin cancer development in humans, it remains unclear whether the chemopreventive activity of COX-2 inhibitors are chiefly attributable to their anti-inflammatory effects. Our study provides evidence that subclinical subepithelial inflammatory foci occur prior to overt tumor formation, and that these areas are highly predictive for future tumor formation, that celecoxib's ability to suppress tumorigenesis is tightly linked to its ability to reduce the area of subclinical inflammatory foci.

8926-40, Session 8

Polarization enhanced multispectral wide-field imaging for evaluating dermal structural changes caused by non-ablative fractional laser treatment

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Non-ablative fractional treatment (NAFT) with mid-infrared lasers is a well-established skin rejuvenation technique. Even though clinical efficacy of this regimen has been demonstrated in several studies, dynamics of changes in skin structure due to this treatment is not fully understood. In this study, we introduced a polarization enhanced multispectral wide-field reflectance imaging method to monitor the effect of this treatment on skin collagen. 6 volunteers belonging to 3 age groups used a NAFT device (PaloVia, Cynosure Inc.) according to recommended daily treatment regimen for two weeks. Wide-field reflectance images of both co-polarization and cross-polarization were acquired between 390 and 750 nm for each volunteer before and after the treatment. Images were then analyzed with proprietary software. Collagen density, full width at half maximum (FWHM) of the intensity histogram and average pixel value of the collagen area were determined. Quantitative results revealed increase in collagen content after the treatment. Our noninvasive in vivo imaging technique is a convenient tool, and is able to monitor dermal structural changes at early stages when clinical results are not yet apparent.

8926-41, Session 8

Hyperspectral imaging for melanoma screening

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The 5-year survival rate for patients diagnosed with Melanoma, a deadly form of skin cancer, in its latest stages is about 15%, compared to over 90% for early detection and treatment. We present an imaging system and algorithm that can be used to automatically generate a melanoma risk score to aid clinicians in the early identification of this form of skin cancer. Our system images the patient's skin at a series of different wavelengths and then analyzes several key dermoscopic features to generate this risk score. We have found that shorter wavelengths of light are sensitive to information in the superficial areas of the skin while longer wavelengths can be used to gather information at greater depths. This system has demonstrated much higher sensitivity and specificity than the currently commercialized system in preliminary trials and has the potential to improve the early detection of melanoma.

Conference 8926B: Therapeutics and Diagnostics in Urology

Saturday 1 –1 February 2014

Part of Proceedings of SPIE Vol. 8926 Photonic Therapeutics and Diagnostics X

8926-43, Session 9

Polarized light imaging for localizing the bladder morphological complications in outlet obstruction disease

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Abnormal growth of prostate is common among aging men [1]. Enlargement of prostate leads to bladder outlet obstruction (BOO) which creates excessive pressure in the bladder and may result in irreversible structural damage to the bladder wall, with further muscle dysfunction [1]. The location of these morphological changes cannot be identified with current imaging methods, thus in many cases the bladder has to be removed [1].

Among different animals, rats have been widely used as BOO models since they exhibit similar tissue complication and urodynamics to humans [1]. In this study, we use polarized light imaging to locate the obstruction-induced morphological changes in ex vivo distended rat bladders. Polarized light imaging can noninvasively quantify micro-structural organization and tissue anisotropy in terms of optical birefringence [2].

We have conducted two studies: 2 week obstruction (12 rats) and 6 weeks obstruction (12 rats). Each group was divided into two: obstructed and control (which have undergone surgery without obstruction). All bladders were harvested and distended up to maximum distension pressure of a normal bladder. The Mueller matrix of different regions of each bladder wall was measured in backscattering geometry using polarized light. The Mueller matrices were mathematically analyzed via polar decomposition, and resultant retardance values were derived [2]. Results show that the local retardance (birefringence) of the ventral urethral regions increases significantly due to obstruction, and the increase scales with the bladder mass. The potential of this new polarimetric imaging technique in urology is discussed.

[1] K. Aitken et al, Nat. Rev. Urol. (2009) 6: 596-621.

[2] S. Alali et al, J. Biomed. Opt. (2012) 17:086010.

8926-44, Session 9

Differentiation of testicular tissue and in situ localisation of vital spermatozoa by probe-based confocal laser endomicroscopy (pCLE)

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Introduction and objective:

In the case of azoospermia, testicular sperm extraction (TESE) often is the only option to gain spermatozoa for assisted reproduction. However, the overall positive sperm retrieval rate is merely 50 % because the testicular biopsies are taken by accident and spermatogenesis is very different in various regions of the testis. Up to now, no imaging technique is available to localize spermatozoa in the testis under in vivo conditions. Therefore the aim of our study was to evaluate the use of probe-based confocal laser endomicroscopy (pCLE) for in situ localization of vital spermatozoa.

Material and Methods:

Investigations of testicular tissue and ejaculates were performed by pCLE using the Cellvizio® (Mauna Kea Technologies, France) confocal microprobe ProFlexTM S1500 (diameter 1.5 mm, lateral resolution 3.3 ?m) and the microprobe Ultra Mini O (diameter 2.6 mm, lateral resolution 1.4 ?m). Besides human tissue retrieved from transsexual men (n=9) and human ejaculates from healthy donors (n=9), rat and bull testes were examined. Fluorescent labelling was performed by incubation with either 0.001% fluorescein isothiocynate (FITC), 5% cresyl violet solution (CV), 0,001% TO-PRO®-3 Iodide (642/661) and 0.04 % acriflavine (AF). pCLE imaging was correlated to images obtained by confocal laser microscopy, light microscopy and phase contrast microscopy.

Results:

pCLE enabled to visualize spermatozoa, spermatocytes and spermatogonia in the seminiferous tubules of the testes. In ejaculates motile spermatozoa were clearly identified. Labelling was specific for each fluorescent dye, as CV stained intercellular matrix in the tubules and AF predominantly labelled the nuclei of all cells during spermatogenesis.

Conclusions:

pCLE is a valuable imaging technique for testicular cells and for the detection of vital spermatozoa in testes. In vivo visualisation of spermatozoa during TESE might improve positive sperm retrieval rates and therefore might become a valuable tool in the treatment of azoospermic suffering men.

8926-45, Session 9

Optical diagnosis of testicular torsion in children

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Acute spermatic cord torsion or testicular torsion is an acute scrotal condition in children and adolescent that is considered as a surgical emergency due to the risk of testicular ischemia and loss. Once strong clinical suspicion of spermatic cord torsion exists, emergent surgical exploration is mandatory. Although surgical exploration of any acute scrotum has been recommended to prevent or confirm torsion, with increased sensitivity and accuracy of diagnostic modalities most surgeons nowadays prefer to investigate and select patients requiring surgery.

We report a case of testicular torsion in a toddler that was diagnosed using a noninvasive optical method. Patient was a 14-month old boy who referred to our center following 24 hours of left scrotal wall swelling erythema and tenderness associated with nausea and emesis but no fever. Physical examination confirmed unilateral scrotal erythema and swelling and identified left testicular tenderness and an intact cremasteric reflex. Urine analysis was normal. Color Doppler ultrasonography had equivocal findings with trend to epididymitis. We used a spatially resolved near infrared spectroscopy (SR-NIRS) device to study and compare tissue oxygen saturation index (TSI%) on both right and left spermatic

cord. TSI% was significantly reduced in the left side (68.8% vs. 75.6%). Both testicles were surgically explored and left testis was found non-viable with a 180-degree intravaginal torsion.

This observation suggests that SR-NIRS monitoring of spermatic cord oxygen saturation appears feasible as a non-invasive, bedside and quick optical method to identify testicular torsion.

8926-46, Session 10

Rapid infrared laser sealing and cutting of porcine renal vessels, ex vivo

Nicholas C. Giglio, Thomas C. Hutchens, William Perkins, The Univ. of North Carolina at Charlotte (United States); Cassandra Latimer, Arlen K. Ward, William H. Nau, Covidien (United States); Nathaniel M. Fried, The Univ. of North Carolina at Charlotte (United States)

Introduction: Suture ligation and cutting of blood vessels during surgery is time-consuming and skill-intensive. Energy-based, electrosurgical and ultrasonic devices have replaced sutures and mechanical clips (foreign objects in the body), providing rapid hemostasis during surgery. However, some of these devices require separate mechanical blades for cutting, and may create undesirably large collateral zones of thermal damage and tissue necrosis. We are exploring infrared lasers as alternative energy sources for these applications. In previous studies, a 1470-nm laser sealed vessels of 1-6 mm in 5 s, yielding burst pressures of ~500 mmHg. This study utilizes optimized parameters to provide faster sealing and cutting of vessels.

Methods: A 110-Watt, 1470-nm laser beam was transmitted through a fiber and beam shaping optics, producing a linear beam 4.0 mm by 10 mm for sealing, and 1.5 mm by 10 mm for cutting. A two-step process sealed and cut ex vivo porcine renal arteries (1-8 mm diameter) in a benchtop setup. Seal and cut times were 1.0 s each. A burst pressure system measured resulting seal strength, and gross thermal damage measurements were also recorded.

Results: All blood vessels tested (n=30) were sealed and cut, with total irradiation times of 2.0 s, mean burst pressures >1200 mmHg (10 times systolic blood pressure of 120 mmHg), and mean combined seal/collateral thermal coagulation zones of ~3 mm.

Conclusions: An optical-based system is capable of precisely sealing and cutting a wide range of porcine renal vessels, and may provide an alternative to RF and ultrasound-based vessel sealing devices.

8926-47, Session 10

Blood coagulation using high intensity focused ultrasound (HIFU)

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High Intensity Focused Ultrasound (HIFU) technology provides a feasible method of achieving thermal coagulation during surgical procedures. One of potential clinical benefits of HIFU could induce immediate hemostasis without suturing. The objective of this study was to investigate the efficiency of HIFU system for blood coagulation of severe vascular injury. HIFU treatment was implemented immediately after bleeding. The ultrasound probe was constructed from piezoelectric material, high-power, generating a central frequency of 2.0 MHz as well as an ellipsoidal focal spot of 1 mm in lateral dimension and 9 mm in axial dimension. Acoustic coagulation was employed on a perfused rabbit artery model in vitro. A surgical incision, (1 to 2 mm long), was made with a scapel on the arterial wall, and heparinized autologous blood was made to leak out from the incision with a syringe pump. A total of 10 incisions was treated with the HIFU beam at a treatment speed of 1 mm/s. The intensity at the focus ranged from 2500 to 3000 W/cm2 for all treatments. Complete hemostasis was achieved in 9 treatments (90%) out of 10 treatments, along with the treatment times of 5 to 10 seconds. The estimated intraoperative blood loss was from 2 to 10 mL. The proposed HIFU system may provide an effective method for immediate blood coagulation for arteries and veins in clinical applications.

8926-48, Session 10

Ex vivo evaluation of safety and efficacy of vaporization of the prostate

using a 300 W high-power laser diode with the wavelength of 980 nm

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Laser vaporization of the prostate is one of the promising technique for less-invasive treatment of benign prostatic hyperplasia. However, shorter operative duration and higher hemostatic ability are expected. The wavelength of 980 nm offers a high simultaneous absorption by water and hemoglobin, so that it combines the efficient vaporization with good hemostasis. Therefore, we have evaluated the safety and efficacy of vaporization of the prostate using a recently developed 300 W high-power laser diode with the wavelength of 980 nm. First, validity of the bovine prostate tissues as the sample was confirmed by measuring the optical properties of the bovine and human prostate tissues using a double integrating sphere optical system. Next, contact and non-contact ex vivo irradiations were performed for various irradiation powers and times, and vaporized and coagulated depths were measured. In the contact irradiation, the vaporized depth at the power of 300 W was significantly deeper than that at the power of 100 W, while the difference was relatively smaller for the coagulated depths at 300 and 100 W. In the non-contact irradiation, coagulation as thick as that in the contact irradiation was observed almost without vaporization. Therefore, it is suggested that the treatment in the contact irradiation using the high-power laser diode can vaporize the prostate more efficiently without increasing the risk of perforation. Hemostasis with the coagulation would be possible in both contact and non-contact irradiations. To prevent the postoperative perforation, operators need to understand the relationship between the coagulated depth and the irradiation conditions.

8926-49, Session 10

Intraluminal occlusion of the seminal duct by laser and histoacryl: two non-invasive alternatives for vasectomy

Benjamin Freitag, Klinikum der Univ. Münich (Germany); Ronald Sroka, Laser-Forschungslabor (Germany); Sabine Koelle, Univ. College Dublin (Ireland); Armin J. Becker, Wael Khoder, Klinikum der Univ. Münich (Germany); Thomas Pongratz, Laser-Forschungslabor (Germany); Christian G. Stief, Matthias Trottmann, Klinikum der Univ. Münich (Germany)

Introduction and objective:

Vasectomy is a well-established method in family control. Even though it is a safe and low risk operation, this surgery is invasive and difficult to reverse. Therefore the aim of this study was to investigate new non-invasive methods for occlusion of the seminal duct.

Material and Methods:

Seminal duct tissue was obtained from patients (n=30) suffering from prostate cancer and therefore undergoing prostatectomy. In a first set of experiments, the seminal duct was occluded by intraluminal application of 1 mL Histoacryl® (Braun Aesculap AG, Tuttlingen, Germany). In a 2nd

set of experiments, endoluminal laser induced occlusion was performed; Four different laser wavelengths (1940 nm, 1470 nm, 1064 nm, 940 nm) and different sets of laser parameters (e.g. power, exposure duration, fibre diameter, applied energy) were compared. Additionally Histoacryl® treated probes were re-opened by means of the laser. Effectiveness of occlusion of the seminal duct was proven by post-treatment irrigation flow measurement, as well as by morphological analyses. To evaluate a potential damage of the surrounding tissue, external temperature was measured using a thermometer during laser application.

Results:

Intraluminal application of Histoacryl® induced an immediate and complete occlusion of the seminal duct. The epithelium and the underlying connective tissue maintained their functional integrity after this treatment. Laser light application to the Histoarcyl® block resulted in a re-opening of the lumen. Treatment with laser energy regularly resulted in shrinkage of the ductal lumen. The laser application generally caused necrosis in the epithelium and induced formation of vacuoles in the underlying connective tissue. As described for endoluminal varicosis treatment, this distinct local reaction might result in an intense inflammation leading to a functional occlusion of the vas deferens.

Conclusions:

Both laser-induced occlusion and application of Histoacryl® are fast and simple techniques which may be able to achieve a functional occlusion of the seminal duct. The application of Histoacryl® additionally may be easily reversible by laser treatment.

8926-50, Session 10

Investigation on the smoke development during laparascopic surgery

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A special container set-up was created to collect the laser induced smoke. Smoke was suctioned through a capillary. The amount of light scattered by the smoke particles when flowing through this capillary was measured. Different laser parameter were used. Additional optical paramter were measured of the test tissue. The vaporized tissue volume was measured. Light scattering, optical parameters and vaporized tissue volume were correlated.

Measurement showed reproducible result. IR wavelengths showed reduced smoke development compared to NIR wavelengths. The appearance of smoke depends on the wavelength. The clinical relevance will be presented.

8926-51, Session 10

A compact, inexpensive infrared laser system for continuous-wave optical stimulation of the rat prostate cavernous nerves

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Introduction: Optical nerve stimulation (ONS) has been commonly performed in the laboratory using high-power Holmium:YAG, diode, and Thulium fiber lasers. However, the relatively high cost of these infrared (IR) lasers in comparison with conventional electrical nerve stimulation (ENS) equipment remains a significant barrier to widespread adoption of ONS. Subsurface ONS of the prostate cavernous nerves (CN's) has recently been reported using lower cost, continuous-wave (CW), all-fiber-based diode laser systems. This study describes further miniaturization and reduction in cost of the ONS system in the form of a compact, lightweight, cordless, and inexpensive handheld infrared laser.

Methods: A 140-mW, 1550-nm diode laser was integrated with a low-power green aiming beam and collimating optics into a compact design. Surface and subsurface ONS was performed in a total of 5 rats, in vivo, by first transplanting a thin layer of testicular fascia over the CN's to simulate the human anatomy, and then measuring an intracavernous pressure (ICP) response during CW laser irradiation with a spot diameter of ~1 mm for 30 s.

Results: Subsurface ONS of the rat CN's was achieved with a threshold incident power of ~75 mW, ICP response time as short as ~6 s, and signal-to-noise ICP ratios of up to 3:1.

Conclusions: Short-term, CW ONS of the prostate CN's is feasible using a compact, lightweight, inexpensive, and cordless IR laser diode system. This system may represent a low-cost, noncontact alternative to ENS for laboratory studies, and with further development, a handheld option for ONS in the clinic to identify and preserve the prostate CN's during prostate cancer surgery.

8926-52, Session 11

Characterization of a 50-um-core optical fiber for potential use in Thulium fiber laser lithotripsy

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Introduction: The Thulium fiber laser's (TFL) single-mode spatial beam profile allows coupling of high laser power into small fibers. Although small fibers have a greater tendency to suffer from distal tip "burn-back", they may also provide increased saline irrigation rates through the ureteroscope working channel, improved ureteroscope deflection, and reduced stone retropulsion during lithotripsy. In this study, an ultra-small, 50-?m-core-diameter, 85-?m-outer-diameter fiber is characterized for TFL vaporization of urinary stones, ex vivo.

Methods: A TFL with wavelength of 1908 nm, pulse energy of 35 mJ, pulse duration of 500 ?s, and pulse rate of 50 Hz was used. Fiber transmission and burnback tests, stone ablation and retropulsion studies, and ureteroscope deflection and irrigation rate measurements were performed.

Results: The 50-?m fiber delivered up to 15.4 ± 5.9 W under extreme bending (5-mm-radius). Ureteroscope working channel flow rates including the fiber decreased by only 10% with no impairment of ureteroscope deflection. Stone retropulsion was minimal and fiber burnback was significant, as expected. Stone vaporization rates measured 70 ± 22 ?g/s using standard pulse trains at 50 Hz, and 99 ± 29 ?g/s using dynamic pulsing methods (5 pulse trains at 10 Hz).

Conclusions: The 50-?m-core fiber may be used as a trunk fiber for TFL lithotripsy while consuming less than 30 times the cross-sectional area of a standard 270-?m-core medical fiber in the ureteroscope's working channel, for enhanced saline irrigation and ureteroscope deflection. With further development, TFL lithotripsy using ultra-small, 50-?m-core fibers may introduce new possibilities for integration with stone baskets and ureteroscopes.

8926-53, Session 11

Investigation on the impact of pulse duration for laser induced lithotripsy

Ronald Sroka, Thomas Pongratz, Tugba Kiris, Sebastian Fiedler, Laser-Forschungslabor (Germany)

In-vitro investigation of Ho:YAG-laser induced stone fragmentation have been performed to identify potential impacts when different pulse durations are available.

After measuring the pulse duration at the end of the bare fiber of a newly developed Ho:YAG-laser system providing a long (LP) and a short pulse (SP) mode, fragmentation tests on artificial BEGO-Stones, drilling tests through a defined BEGO-layer and fiber burn back tests were performed under reproducible experimental conditions. Additionally, the repulsion of long versus short laser pulses was compared using the pendulum set-up.

The pulse duration depends on the laser parameter used. While the pulse duration at pulse energy of 2J/pulse was about 2.5-times longer in the LP-mode compared to the SP-mode, at 0.5J/pulse the difference is about a factor of 1 to 1.5. Fragmentation rates differ slightly when comparing the two pulse duration regimes. The drilling test showed a significant 4 to 6 times faster drilling in case of SP compared to LP. Using LP the fiber burn back is nearly negligible while SP showed an increased burn back. The results of the pendulum test showed that the deviation induced by the momentum of SP is about 1.5times increased compared to LP.

In conclusion LP-mode showed reduced side effects like repulsion and fiber burn back in comparison to SP-mode while the potential for fragmentation looks not significant different, thus a more convenient handling during operation seems achievable.

8926-54, Session 11

Rapid vaporization of kidney stones, ex vivo, using a Thulium fiber laser at pulse rates up to 500 Hz with a stone basket

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Introduction: Previous studies have shown that the Thulium fiber laser (TFL) is capable of ablating kidney stones. However, it is unclear whether TFL lithotripsy, when limited to low pulse energies, can ablate stones at rates sufficient for future clinical use. This study characterizes TFL lithotripsy at low pulse energies and ultra-high pulse rates for rapid ablation of stones stabilized by a clamp or basket.

Methods: TFL energy at 1908 nm, 35 mJ, and 500 microseconds was delivered through 100-micron-core fibers in contact with uric acid (UA) and calcium oxalate monohydrate (COM) urinary stones, fixed and submerged in saline. Standard pulse trains at 10-500 Hz and customized pulse packets (5 or 10 micropulses in each macropulse packet) were compared. Stone mass loss was measured and ablation rates calculated, and fiber burn-back was recorded for each study (n>5).

Results: Fiber burnback was ~0.1 mm for UA stones, and up to ~7 mm for COM stones after 1 min. Stone vaporization rates up to 5.0 mg/s and 1.3 mg/s were measured for UA and COM stones, respectively, at 500 Hz. Transition from stone “vaporization” with dust sized particulates to stone “fragmentation” with >1 mm particles occurred at pulse rates >200 Hz. At low pulse rates, pulse packets provided a 2-fold increase in vaporization rates, however at high pulse rates, conventional and micro-pulse trains vaporization rates were equivalent.

Conclusions: Use of ultra-high pulse rates and dynamic pulsing during TFL lithotripsy results in stone vaporization rates that may be practical for future clinical use.

8926-55, Session 11

Characterization of calculus migration during Ho:YAG laser lithotripsy by high speed camera using suspended pendulum method

James J. Zhang, Danop Rajabhandharaks, Jason R. Xuan, Ray W. Chia, Tom Hasenberg, American Medical Systems (United States)

Calculus migration is a common problem during ureteroscopic laser lithotripsy procedure to treat urolithiasis. A conventional experimental method to characterize calculus migration utilized a hosting container (e.g. a “V” grove or a test tube). These methods, however, demonstrated large variation and poor detectability, possibly attributing to friction between the calculus and the container on which the calculus was situated. In this study, calculus migration was investigated using a pendulum model suspended under water to eliminate the aforementioned friction. A high speed camera was used to study the movement of the calculus which covered zero order (displacement), 1st order (speed) and 2nd order (acceleration). A commercialized, pulsed Ho:YAG laser at 2.12 um, 365-um core fiber, and calculus phantoms (Plaster of Paris, 10?10?10mm cube) were utilized to mimic laser lithotripsy procedure. The phantom was hung on a stainless steel bar and irradiated by the laser at 0.5, 1 and 1.5J energy per pulse at 10Hz for 1 second (i.e., 5, 10, and 15W). Movement of the phantom was recorded by a high-speed camera with a frame rate of 10,000 fps. Maximum displacement was 1.86±0.09, 3.28±0.65, and 4.79±0.46 mm for 0.5, 1, and 1.5J energy per pulse, respectively. Using the same laser power, the conventional method showed <0.5 mm total displacement. When reducing the phantom size to 5?5?5mm (1/8 in volume), the displacement was very inconsistent. The results suggested that using the pendulum model to eliminate the friction improved sensitivity and repeatability of the experiment. Detailed investigation on calculus movement and other causes of experimental variation will be conducted as a future study.

8926-56, Session 11

An integrated fiber and stone basket device for use in Thulium fiber laser lithotripsy

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Introduction: The Thulium fiber laser (TFL) is being explored as an alternative laser lithotripter to Holmium:YAG laser. The TFL’s Gaussian beam profile provides high power transmission through small-core fibers with reduced proximal fiber tip damage. Recent studies have also reported that attaching hollow steel tubing to the fiber decreases distal fiber tip degradation/burn-back at high laser pulse rates without compromising stone ablation rates. However, increased stone retropulsion was still observed. In this study, the hollow steel tip fiber is integrated into a stone basket to provide minimal stone retropulsion during ablation.

Methods: A device was constructed of 100-?m-core, 140-?m-OD, low-OH, silica fiber outfitted with 1-cm-long steel tubing at the distal tip, and permanently integrated with a 1.3-Fr (0.433-mm-OD) disposable nitinol wire basket, to form a 1.9-Fr (0.633-mm-OD) design. This compact design allows for increased irrigation rates through the ureteroscope working channel compared to separate manipulation of fiber and stone basket, and currently available 2.4-Fr (0.800-mm-OD) baskets.

Results: TFL pulse energy of 35-mJ with 500-?s pulse duration and variable pulse rates (100-500 Hz) were delivered through the integrated fiber/basket device in contact with COM and UA stones for successful stone ablation during ex vivo studies. This design also provided increased irrigation rates compared to conventional baskets.

Conclusions: A compact and integrated fiber / stone basket device was successfully tested for TFL lithotripsy. With further development, this device may be used in ureteroscopes providing not only stone stabilization, but also increased irrigation rates and improved ureteroscope deflection during TFL lithotripsy.

8926-57, Session 11

Visualizing mechanical stress, pressure waves and liquid flow during laser lithotripsy

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The mechanism of action of the holmium laser lithotripsy is attributed to explosive expanding and imploding vapor bubbles in association with high speed water jets creating high mechanical stress and cracking the stone surface. A good understanding of this mechanism will contribute to the improvement and the safety of clinical treatments. Two new methods have been developed to visualize the dynamics of mechanical effects and fluid flow induced by Holmium laser pulses around the fiber tip and the stone surface. The fiber tip was positioned near the surface of a stone submerged in either beer or water. In beer, at the moment of a laser pulse, a cloud of carbon dioxide micro bubbles was formed representing the area of instant pressure change inducing mechanical stress. The effects were captured with high speed imaging at 2000 f/s. After the pulse, the dynamics of the bubble cloud flow could be appreciated. In water, the dynamics of the pressure wave after the pulse could be visualized by observing the optical deformation of a fine grid pattern in the background of the water container using digital subtraction software. The micro bubbles showed the locations of the highest mechanical stress and turbulent flows along the surface of the stone. These new imaging techniques provide a good understanding of the mechanical effects contributing to the effectiveness and safety of lithotripsy and can be used to study the optimal fiber shape and position towards the stone surface.

8926-58, Session PSat

In vitro evaluation of the effect of laser irradiation time on tissue ablation depth

Aditi Ray, Thuy T. Nguyen, Danop Rajabhandharaks, Hui Wang, Ray W. Chia, Tom Hasenberg, American Medical Systems (United States)

Laser surgical treatment of benign prostatic hyperplasia (BPH) involves focused delivery of laser energy through a flexible fiber on the target tissue. Although not encountered often, accidental bladder perforation during the procedure remains a safety concern. A previous study (Kang et al. Lasers in Medical Science 2012) reported that bladder perforation was unlikely in the event of forward propagation of laser light. However the study tested only at power levels of 80W and 120W. The present study looked at the effect of laser irradiation time on tissue ablation at higher power (180W). Two different power levels were tested (120W and 180W) using side-firing fibers of either 600µm or 750µm diameter on porcine kidney samples. The resulting ablation depth (N=10) was measured at each irradiance time (5s, 10s, 30s, 60s) and power level. Preliminary results showed that the ablation depth created at 120W using the 600µm fiber (5s:11.91±2.42mm; 10s:13.71±3.03mm; 30s:16.31±5.42mm; 60s:17.38±5.96mm) was higher than that created at 180W with the 750µm fiber (5s:9.21±1.61mm; 10s:11.15±1.03mm; 30s:14.83±1.79mm; 60s:15.90±2.25mm). However this difference was not statistically significant ($p>0.05$). An earlier study has demonstrated the higher vapourization efficiency at 180W (Kang et al. Journal of Urology 2010). Therefore it can be concluded that tissue ablation at 180W with a 750µm diameter fiber can be achieved with higher vaporization efficiency without penetrating deep inside the tissue. Hence accidental perforation during laser prostatectomy is unlikely even at high power of 180W.

8926-59, Session PSat

Effect of working distance on soft tissue in vitro during 532nm and 2.1µm laser prostatectomy

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Distance between target tissue location and fiber delivery device (i.e., working distance) can significantly affect tissue ablation characteristics during laser prostatectomy. In this study we investigated the effect of working distance on soft tissue in vitro between 532nm and 2.1µm lasers using a porcine kidney model. Fiber delivery devices and tissue samples were secured on an automated ablation station to mimic laser prostatectomy. Average power of 100W and 120W was utilized for 2.1µm and 532nm, respectively. Tissue ablation characteristics including tissue ablation volume (AV), ablation depth (AD), and coagulation thickness (CT) were measured and compared between 532nm and 2.1µm lasers at working distances (WD) of 1, 2, 3, and 8mm. The results showed that AV and AD decreased as WD increased for both lasers. This could be due to reduced radiant exposure resulting from increasing WD. Overall, AV532nm was 60-70% larger than AV2.1µm at the same WD. AV2.1µm became negligible at 8mm WD. CT generated by 532nm laser increased as a function of WD (CT532nm=0.78±0.11, 0.81±0.12, 0.92±0.14, and 1.12±0.19mm at WD=1, 2, 3, and 8mm). However, unlike 532nm laser, CT decreased as WD increased for 2.1µm laser (CT2.1µm=0.79±0.11 and 0.59±0.11mm at WD=1 and 2mm. CT2.1µm was negligible at WD≥3mm). One possible reason for this could be due to the effect of the shock wave generated from the cavitation collapse on tissue removal. This effect is secondary to the absorption of the 2.1µm laser in water and can thus lead to mechanically removing the tissue with minimal thermal effects. As a result, a thinner CT may be observed with increasing WD. In conclusion, the two lasers produced different tissue ablation characteristics as a function of working distance. Thus proper control of the working distance as recommended by the two lasers is crucial for optimal surgical outcomes during laser prostatectomy.

8926-60, Session PSat

A quantitative study on ICG-conjugated single-walled carbon nanotubes for photoacoustic imaging of vesicoureteral reflux

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Single-walled carbon nanotubes (SWNTs) conjugated with indocyanine green (ICG) have frequently been used with photoacoustic tomography (PAT) to detect bladder diseases such as vesicoureteral reflux (VR). However, the efficacy and safety of SWNTs-ICG in living bladders have not been quantitatively assessed unlike indocyanine green (ICG) and methylene blue (MB). The objective of the current study was to demonstrate the threshold level of SWNTs-ICG for PAT-assisted VR diagnosis in light of concentrations and post-injection time. In a phantom study, SWNTs-ICG with different concentrations from 0 to 300 nM was photoacoustically imaged at various wavelengths compared with other contrast agents such as MB, ICG, and SWNTs. To demonstrate

the feasibility of mapping pediatric bladder by using SWNTs-ICG with PAT, rats superimposed by chicken breast tissue, of which thickness was 30±20 mm equivalent to that of bladder tissue, were utilized with 300 nM of SWNTS-ICG. Simultaneously, the time duration after agent injection was confirmed to verify how long SWNTs-ICG could take to flow out. As a result, the sensitivity of SWNTs-ICG was up to four-fold, compared with the other contrast agents at 50 nM and at each peak wavelength (SWNTs-ICG and ICG at 820 nm, MB at 677 nm and no peak with SWNTs). In vivo images strongly evidenced that 100 nM of SWNTs-ICG enabled PAT to map deeply located tissue layers (up to 53 mm) and completely flow out 24 hours after post-injection. Therefore, SWNTs-ICG can be an efficient and safe VR diagnostic tool at low doses due to its high absorption contrast.

8926-150, Session PSat

Multimodal evaluation of the efficacy of ICG-photodynamic therapy to tumors in rabbit

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Indocyanine green (ICG) is a dye that is officially approved for diagnostic purposes. Its absorption peaks in the near infrared (NIR) range, and it was readily attached to plasma protein. Therapeutic ability of ICG with a diode laser has been studied in well-vascularized cutaneous tumors. In this study, we developed a rabbit tumor model and showed the effect of NIR photodynamic therapy (PDT) with intravenous ICG on squamous cell carcinoma in rabbits. ICG (twice, 2 mg/kg each time, intravenously) was injected into the ear veins of four male New Zealand white rabbits with hypervascular tumors. Then, the tumors were irradiated with a diode laser (? = 810 nm, 300 J/cm2,3W). ICG angiography and optical coherence tomography (OCT) were used to evaluate the efficacy of photodynamic therapy. At one week after initial treatment, six of the eight tumor lesions treated showed a response. Within the follow-up period of 2 weeks after the first response, tumor regrowth was noticed in all eight lesions. All tumor lesions showed minimal crust formation and healed within several days. ICG photodynamic therapy is an effective palliative therapeutic modality with a low rate of side effects for the treatment of tumors in rabbit.

8926-42, Session 12

Volumetric mosaicing for optical coherence tomography for large area bladder wall visualization

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Optical coherence tomography (OCT) has shown potential as a complementary imaging modality to white light cystoscopy (WLC) because it can visualize sub-surface details of the bladder wall, enabling it to stage early cancers and visualize tumors undetectable to WLC. However, the inherently small field of view (FOV) of OCT compared with the area of the bladder wall restricts its clinical utility. A large OCT FOV could improve surgical planning by enabling complete visualization of tumor margins or could aid in early cancer detection by tracking the appearance of the bladder wall over time. To overcome the limited FOV of OCT, we present an algorithm for volumetric mosaicing with OCT data. This algorithm can automatically mosaic tens of volumes and accounts for six degrees of freedom between volumes; these characteristics are necessary for imaging large organs with a freehand probe and distinguish the algorithm from prior approaches for OCT mosaicing. We exploit the morphology of the bladder wall and white light information available during WLC. We compute a 2D transformation between sequential WLC images and then utilize this transformation, the relationship between the WLC and OCT data, and the extracted surface from OCT to compute a 3D transformation between volumes. The algorithm is validated qualitatively and quantitatively with ex vivo bladder samples and a custom silicone phantom that provides calibrated image targets and mimics essential features of the bladder wall. The realization of this algorithm is a critical step to enabling OCT to contribute meaningfully to bladder surveillance and surgical guidance.

8926-61, Session 12

Quick and non-destructive qualification of prostate biopsies with full-field OCT

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The diagnosis of prostate cancer involves multiple randomized biopsies, leading to over-diagnosis and over-treatment, as well as unnecessary non-informative samples. Recent studies have shown that MRI-targeted biopsies allowed similar detection performance than randomized biopsies, while decreasing the number of cores. Full-field optical coherence tomography (FFOCT) offers a non-invasive method of obtaining images of biological tissues at ultrahigh resolution (1µm in all 3 directions) approaching traditional histological sections. It could be used to validate the cores just after they are biopsied, in particular when MRI-targeted.

More than 100 prostate biopsies from 28 patients were imaged within minutes with FFOCT before the biopsies were sent to the pathology lab for standard histological assessment. The pathologists were asked to analyze the FFOCT images to set reading criteria and provide a blinded diagnosis. The concordance between the FFOCT diagnosis and the histological diagnosis as well as the learning curve was recorded.

Structures of normal tissue (eg fibro-muscular stroma, adipocytes, and vessels) could be recognized on the FFOCT images. The architectural details enabled to identify tumorous areas in the biopsies in many cases. The most frequent reason for false negatives and false positives were small lesions and hyperplasia respectively.

FFOCT is as a fast and non-destructive imaging technique that provides a quick assessment of the tissue morphology and appears as an additional detection tool for prostate cancer screening. The technique could be used on-site during the biopsy procedure to validate the biopsies and guide the number of biopsies to be performed.

8926-63, Session 12

Native excitation and emission matrix fluorescence spectroscopy for quantification of tissue native fluorophores and cancer diagnosis

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Native fluorescence spectrum of human prostate normal and cancerous tissues is studied to distinguish a) normal and cancerous tissues, b) cancerous tissues at different cancer grade. The tissue samples were obtained from Cooperative Human Tissue Network (CHTN) and National Development and Research Institutes (NDRI). The prostate tissues were processed and fixed in paraffin wax. An excitation and

emission matrix (EEM) is generated for each tissue sample by acquiring native fluorescence spectrum of the sample using multiple excitation wavelengths. The non-negative matrix factorization (NMF) algorithm is used to decompose the EEM fluorescence data for all samples. Non-negative component fluorescence spectra are generated and correspond to the fluorophores in biological tissues, including tryptophan, collagen, elastin, nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD) and the background paraffin. Due to metabolic changes in the in the development of cancer, we hypothesize the concentrations of collagen, NADH and FAD are different in normal and cancerous tissues, and also different for different cancer grade. We used the ratio of the abundances of FAD and NADH to distinguish normal and cancerous tissues, and cancerous tissue at cancer grade. The FAD-to-NADH ratio was found to be the highest for normal tissue and decrease as the cancer grade increases.

8926-64, Session 12

Volumetric in-vivo visualization of upper urinary tract tumors using optical coherence tomography

Daniel M. de Bruin, Academisch Medisch Ctr. (Netherlands)

Purpose: Knowledge on tumor stage and grade is paramount for treatment decision in Upper Urinary Tract Urothelial Carcinoma (UUT-UC), but cannot be accurately assessed by current techniques. Optical Coherence Tomography (OCT) is a technique, which can hypothetically provide the urologist with real-time intra-operative information on tumor grade and stage. In this pilot study the first results of OCT in grading and staging of UUT-UC are presented.

Material & Methods: Eight consecutive patients underwent URS for UUT-UC suspicion or follow-up. OCT datasets were intra-operatively obtained from the ureter and pyelum. All patients eventually underwent nephroureterectomy. OCT staging was performed by visual inspection of lesions found on OCT images and OCT grading by quantification of the OCT signal attenuation (μoct [mm-1]) on lesions and compared with histopathological diagnosis. A Wilcoxon rank sum test was used for statistical analysis.

Results: Seven in-vivo OCT diagnoses on staging were in accordance with histology. In the eight patient tumor thickness transcended OCT imaging depth range and was therefore inconclusive on invasiveness. For grading, median (interquartile range) μoct for grade 2 lesions was 1.97 mm-1 (1.57-2.30) and 3.53 mm-1 (2.74-3.94) for grade 3 (p-value <0.001). Healthy urothelium was too thin to reliable determine μoct.

Conclusions: OCT is a promising minimally invasive tool for real time intra-operative optical diagnostics for tumors in the upper urinary tract. Our study results warrant future research to determine in a larger sample size grading and staging accuracy of OCT and the possible implementation of OCT in the diagnostic algorithm of UUT-UC

8926-65, Session 12

Method for improving photodynamic diagnosis and surgery of bladder tumours in flexible cystoscopes

Lars R. Lindvold, Technical Univ. of Denmark (Denmark); Gregers G. Hermann, Frederiksberg Hospital (Denmark)

At Photonics West 2013 we presented a paper (8565-41) on how to remove unwanted green fluorescence from urine during Photodynamic Diagnostics (PDD) of tumours in the bladder using cystoscopy. This paper was based on spectroscopic observations of urine and the photosensitiser Protoporpyhrin IX (PpIX). A high power LED based light source (525 nm) has been made in our laboratory according to the findings presented in paper 8565-81. This light source is tailored to match most commercially available fibre-based and rigid cystoscopes. A suitable spectral filter and adapter, for the eyepiece of the cystoscope, has been made which allows the urologist to observe both red fluorescence from tumours and autofluorescence from healthy tissue at the same time. In this paper we will present our findings from a series of test on patients(1) in the operating room (OR) and outpatient department (OPD) based on the tailor-made LED light source . The purpose of this test is to demonstrate that PDD can be performed on bladder tumours in the outpatient department using the tailor-made high power LED light source using flexible cystoscopes. The paper will also address the issue of photobleaching of the photosensitiser with respect to the excitation wavelength. Measurements showing the improvement in photosensitiser lifetime and hence extended observation time for the urologist will be presented.

1. The patients were treated in accord to the Helsinki Declaration, the regulations of the local ethical committee and to the Danish guidelines for treatment of bladder cancer.

Conference 8926C: Optical Imaging, Therapeutics, and Advanced Technology in Head and Neck Surgery and Otolaryngology

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8926 Photonic Therapeutics and Diagnostics X

8926-118, Session 1

Quantitative pneumatic otoscopy using low-coherence interferometry in a handheld device

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Primary care physicians and ear health specialists use otoscopy as a tool to evaluate the health of the middle ear. An otoscope allows the physician to examine the tympanic membrane (TM) by magnifying the surface of the tissue. However, this examination is highly subjective, relying on the physician's qualitative description of characteristics such as color, shape and translucency of the TM. Pneumatic otoscopy is a functional extension of traditional otoscopy that allows the physician to qualitatively assess the likelihood of an effusion in the middle ear. Despite the fact that the American Academy of Pediatrics recommends the use of pneumatic otoscopy, many physicians forgo this examination even when an effusion may be present.

Experienced physicians that make routine use of pneumatic otoscopy strongly advocate its benefits for evaluating the presence of fluid in the middle ear. Recognizing the benefit of this technique and the limitations it exhibits in its current implementation, we have developed a method to quantitatively evaluate the movement of the TM under pneumatic stimulus. We have developed an otoscope modified to include low-coherence interferometry (LCI) in order to provide measurements of TM deflection on a 5-10 micron scale. LCI uses harmless, near-infrared light to provide depth-resolved structural images of tissue in real-time. The device is used to measure deflections induced in ear models with and without middle ear effusions, as well as initial in vivo measurements in humans. Specific challenges are discussed, such as motion artifact and extraction of accurate quantitative information.

8926-119, Session 1

Differentiating acute and chronic otitis media with optical coherence tomography in a primary care imaging system

Guillermo L. Monroy, Ryan L. Shelton, Ryan M. Nolan, Cac T. Nguyen, Univ. of Illinois at Urbana-Champaign (United States); Michael A. Novak M.D., Malcolm Hill M.D., Carle Foundation Hospital (United States); Daniel T. McCormick, Advanced MEMS (United States); Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

The accurate diagnosis of otitis media (OM) lies in navigating numerous confounding and qualitative factors that may present during an exam, including patient history and the presentation of infection. We have developed a Primary Care Imaging system that integrates optical coherence tomography (OCT) and video imaging within a MEMS-based handheld scanner. The system was used to investigate micron-scale morphological changes in the human tympanic membrane (TM), such as TM scattering and thickness, and the presence of a biofilm. An IRB-approved study of 34 pediatric patients was completed, consisting of 13 healthy, normal presentations of the middle ear, 12 acute OM cases, and 9 chronic OM cases, as defined by the investigating physician at the time of exam. When the overall thickness was measured (TM and any associated biofilm), the thickness of normal, acute, and chronic infection groups were found to lie in statistically separate groups (normal-acute and normal-chronic: $p < 0.001$, acute-chronic: $p < 0.0016$), which can then be used to differentiate infection states. When the thickness of the TM and biofilm were considered separately in chronic OM, the chronic TM thickness correlated with the normal group ($p = 0.68$), yet was still distinct from the acute group ($p < 0.001$). By correlating physical characteristics with the previously uninvestigated optical properties of the TM, it may be possible to better differentiate the various states of OM infection in vivo to more effectively manage and refer patients based on quantitative data.

8926-120, Session 1

Fast broadband vibration measurement of the human tympanic membrane ex vivo by optical coherence tomography

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Determining sample motion is of crucial importance in different fields such as medical examinations or material testing. In the medical field, vibrations of the tympanic membrane (TM) and the coupled ossicles are of great interest, because they are mainly responsible for our communication. Changes in the structure or the mechanical properties caused by diseases of the TM can alter the vibration patterns of the TM surface and lead to hearing loss and communication impairment. Until now, clinical routine examinations of the TM are mainly performed by visual inspection via otoscopy or functional tests of the TM and ossicles in conjunction via audiometric measurements. A non-invasive and contact-free in vivo investigation of the structural and functional condition of the TM would be a big step forward. Optical coherence tomography (OCT) is a suitable technology for three-dimensional imaging of tissues on the micron scale and phase resolved Doppler OCT is shown to be a feasible method to measure the vibrating TM with a sufficient spatial and frequency resolution. Therefor a freshly excised human temporal bone specimen, showing no pathology, was used and the TM was stimulated with a loudspeaker in a frequency range between 0.4 to 6.4 kHz. The oscillation of the TM was analyzed for a grid of 25 x 25 measurement points located over the whole TM. The measurement time for all frequencies at all 625 points took only 5.3 s, underlining the potential for future in vivo studies. Thus, OCT enables a simultaneous morphological and physiological imaging of the TM.

8926-121, Session 1

Effect of LLLT on the level of ATP and ROS from organ of corti cells

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It is well established that ototoxic antibiotics and acoustic trauma can damage cochlear hair cells and cause hearing loss. Previous studies using transcanal LLLT (Low level laser therapy) showed that LLLT can promote recovery of hearing thresholds and cochlear hair cells. However, its mechanism has not been studied. Aim: To investigate the mechanism of hearing recovery by LLLT. Methods: HEI- OC1 (House ear institute organ of corti) cells were cultured for 18 hours and ototoxicity was induced by gentamicin (GM) treatment to the cells. Cultured cells were divided into 6 groups, No treatment, GM 6.6 mM and GM 13.1 mM, LLLT only, GM 6.6 mM+LLLT and GM 13.1 mM+LLLT cells. Using 808 nm LD laser, 15 mW was irradiated to the cultured cells for 15 min, 4 hours after GM treatment to the cells. ATP was assayed using the ATP assay Kit. ROS was measured using confocal microscope after application of H2DCFDA dye. Results: ATP was decreased in GM 6.6 and 13.1 mM cells, increased in LLLT only cells, GM 6.6 mM+LLLT and GM 13.1 mM+LLLT cells compared to GM 6.6 and 13.1 mM cells. ROS was decreased in no treatment and LLLT only cells, increased in GM 6.6 mM and GM 13.1 mM cells, and decreased in GM 6.6 mM+LLLT and GM 13.1 mM+LLLT cells compared to GM 6.6 and 13.1 mM cells immediately after laser irradiation and 1 hour later. Conclusion: This study demonstrated that LLLT on GM treated HEI-OC1 cells increased ATP and decreased ROS that may contribute to the recovery of hearing.

8926-122, Session 1

3D optical coherence tomography image registration for guiding cochlear implant insertion

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In cochlear implant surgery, an electrode array is inserted into the cochlear canal to restore hearing to a person who is profoundly deaf or significantly hearing impaired. One critical part of the procedure is the insertion of the electrode array, which looks like a thin wire, into the cochlear canal. Although X-ray or computed tomography (CT) could be used as a reference to evaluate the pathway of the whole electrode array, there is no way to depict the intra-cochlear canal and basal turn intra-operatively to help guide insertion of the electrode array. Optical coherent tomography (OCT) is a highly effective way of visualizing internal structures of cochlea. Swept source OCT (SSOCT) having center wavelength of 1.3 micron and 2D Galvonometer mirrors was used to achieve 7-mm depth 3-D imaging. Graphics processing unit (GPU), OpenGL, C++ and C# were integrated for real-time volumetric rendering simultaneously. The 3D volume images taken by the OCT system were assembled and registered which could be used to guide a cochlear implant. We performed a feasibility study using both dry and wet temporal bones and the result is presented.

8926-123, Session 2

A study of pediatric vocal fold maturation using optical coherence tomography

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Our understanding of the vocal fold development comes from histopathological assessment of cadaveric specimens and is therefore limited by the lack of pediatric samples and by post mortem artefacts. A non-invasive microscopy modality would allow us to ameliorate our understanding of the development of the voice apparatus, which could in turn lead to treatments that are more suited to the pediatric population.

To this end, we investigate the use of optical coherence tomography (OCT) as a clinical tool to provide a qualitative as well as a quantitative description of the layered structure of the lamina propria. We designed a high-resolution OCT hand held probe suitable for longitudinal studies from neonates to adults. Our design is based on relaying the 3D scan of a pair of galvanometer-mounted mirrors to the sample plane through an IR coated grin assembly. A 45° prism is mounted at the distal end of the instrument to observe the free edge of the vocal cord. For sterilization purposes a stainless-steel cover including a side window at its tip is added. A fluorinated ethylene propylene (FEP) sheet insures that the scope is watertight and optically transparent. The hand held unit is coupled to a swept-source OCT system (?0=1310nm, ??=100nm) providing axial and lateral resolutions of 12 and 25 microns respectively. The probe is easily integrated into a clinical setting through attachment to a conventional surgical articulated arm. Our pilot study involves a cohort of 10 patients ranging from ages 8 to 17 years old.

8926-124, Session 2

Automated working distance adjustment for a handheld OCT-Laryngoscope

Sabine Donner, Sebastian Bleeker, Tammo Ripken, Alexander Krueger, Laser Zentrum Hannover e.V. (Germany)

Optical coherence tomography (OCT) is an imaging technique which enables diagnosis of vocal cord tissue structure by non-contact optical biopsies rather than invasive tissue biopsies. For diagnosis on awake patients OCT was adapted to a rigid indirect laryngoscope. The working distance has to match the probe-sample distance and varies from patient to patient. Therefore the endoscopic OCT sample arm has a variable working distance of 40 mm to 80 mm. Automatic working distance adjustment is based on image processing to identify the current axial position. The OCT reference plane and the focal plane of the sample arm are moved according to position errors. Repeated position adjustment during the whole diagnostic procedure automatically keeps the axial position of the sample in the B-Scan at the predefined height. The autofocus identifies and adjusts the working distance within the range of 40 mm within maximum 2.7 s. The continuous image stabilisation reduces the appearance of axial sample movement in the cross-sectional images for handheld OCT scanning. Rapid autofocus reduces duration of the diagnostic procedure and axial position stabilisation eases the use of the OCT laryngoscope. Therefore this work is an important step towards integration of OCT into indirect laryngoscopes.

8926-125, Session 3

Self-motion-tracking probe for long range optical coherence tomography using micro beam splitter

Jiawen Li, Jun Zhang, Alex Wang, Joseph Jing, Zhongping Chen, Univ. of California, Irvine (United States)

Long range optical coherence tomography (OCT), with its high speed, high resolution, non-ionized properties and cross-sectional imaging capability, is suitable for upper airway lumen imaging. To render 2D OCT datasets to the true 3D anatomy, additional tools are usually applied, such as X-ray guidance or a magnetic sensor. X-ray increases ionizing radiation. A magnetic sensor either increases probe size or requires an additional pull-back of the tracking sensor through the body cavity. In order to overcome these limitations, we present a novel tracking method using a 1.5 mm*1.5mm 90/10-ratio micro beam splitter and two marker lines on the probe sheath. 10% light through the beam-splitter is used for motion tracking. 90% light is used for regular OCT imaging and motion

tracking. Two signals corresponding to these two split-beams that pass through different optical path length delays are obtained by the detector simultaneously. Using the two split beams' returned signals from the same marker line, the 2D inclination angle of each step is computed. By calculating the 2D inclination angle of each step and then connecting the translational motion, we can obtain the 2D motion trajectory of the probe. With two marker lines on the probe sheath, 3D inclination angles can be determined and then used for 3D trajectory reconstruction. We demonstrate the feasibility of this design for true 3D structure reconstruction using a porcine trachea specimen. This optical-tracking probe has the potential to be made as small as an outer diameter of 1.0mm, which is ideal for upper airway imaging.

8926-126, Session 3

Multi-scale and functional microscopy for head and neck tissue identification

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Specific tissue identification during thyroid surgery is essential to ensure patient safety and maximize tissue preservation. Surgical interventions rely on intra-operative visual inspection as well as conventional histological analysis of frozen tissue sections to identify small glands and lymph nodes using cellular patterns. Clinical practice can be improved using optical techniques to localize the laryngeal nerve and avoid iatrogenic removal of or damage incurred to parathyroid glands.

We present a multi-scale, multimodal microscope combining two morphological imaging modalities- one with low magnification and one with high magnification with a functional imaging modality imaging near infrared autofluorescence of head and neck tissues. Optical coherence tomography (OCT) performed at 1300nm acts as a low magnification, cross-sectional imaging technique facilitating gland identification through the capsule. Reflectance confocal microscopy (RCM) at 780nm is coupled to OCT to perform higher magnification images with sub-cellular resolution. RCM's optical sectioning yields real-times images comparable to histological sections albeit without the specific contrast provided by staining techniques. Fluorescence imaging adds that contrast for the detection and identification of parathyroid glands [Paras et al., JBO, 2011].

Imaging with all three modalities is performed at video-rate. A dual-band wavelength-swept laser (centered around 780nm and 1310nm) specifically designed for this application was developed and implemented. We present images of the three co-registered modalities acquired simultaneously.

8926-127, Session 3

Miniaturization does not impair the diagnostic value of ESS in human thyroid nodules

Jennifer E. Rosen, Nicholas J. Giordano, Faris Azar, Eladio Rodriguez-Diaz, Ousama M. A'Amar, Irving J. Bigio, Stephanie L. Lee, Boston Univ. (United States)

Introduction: Thyroid nodules are common, but rarely malignant. The current gold standard for diagnosis, fine-needle aspiration (FNA) biopsy yields 10-25% of indeterminate cytology results leading to patients undergoing thyroidectomy for diagnosis. We assessed the technical potential of a miniature elastic scattering spectroscopy (ESS) probe that was designed to fit into a 23 gauge FNA needle assembly to differentiate benign from malignant thyroid nodules. We hypothesized that through miniaturization, we could increase the clinical utility of ESS by pre-operatively diagnosing thyroid nodules.

Methods: We collected data in vivo using the miniaturized ESS probe on patients undergoing ultrasound-guided thyroid biopsy. Using pathology as our gold standard, spectra from the miniaturized ESS probe were compared to previous ESS data that utilized a larger optical geometry to take post-surgical measurements on thyroid nodules.

Results: 136 patients were enrolled. The major consequence of miniaturization was an increased acquisition time, however all measurements on the in vivo system were under one second. ESS features were comparable both between in vivo and ex vivo probes and discrimination of benign from malignant thyroid nodules was noted.

Conclusion(s): An in vivo trial of an invasive miniaturized integrated ESS biopsy probe for discrimination of benign from malignant thyroid nodules is feasible and comparable to larger ex vivo probes. Collection of ESS data during a biopsy would be both practical and reliable. With the development of a disease-specific algorithm, ESS could potentially be used as an in-situ real-time intra-operative diagnostic tool or as a minimally invasive adjunct to conventional FNA cytology.

8926-128, Session 3

Characterizing fluorescent imaging properties of antibodies conjugated to IRDye800CW for use in imaging of head and neck cancer

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Introduction: Proteins conjugated to the near infrared (NIR) moieties for detection of head and neck cancers are being translated to the clinic. However, little is known about the fluorescent properties of IRDye800CW after conjugation to antibodies. We investigated factors that may alter the real-time observed fluorescence of antibody conjugated dye and the rate of fluorescent signal loss.

Methods: Dye fluorescence was examined using three FDA approved antibodies conjugated to IRDye800CW (LI-COR) over a period of 15 days. Temperature effects on fluorescence were examined for conjugated dye in both solution and a mouse tumor model. Samples were cooled to -20°C then warmed to predetermined temperatures up to 60°C with imaging performed using the PEARL Impulse (LI-COR) and LUNA (Novadaq) systems.

Results: Short term loss of fluorescence (< 1 hour) was linear, while long term loss (15 days) was exponential with significantly greater signal loss seen with light exposure and increased temperatures. Cooling of tumor tissue at -20°C was shown to significantly increase tumor fluorescence on both imaging modalities when compared to room temperature ($p=0.008$, $p=0.019$). Concurrently the ratio of tumor to background fluorescent signal (TBR) increased with decreasing temperature with statistically significant increases seen at -20°C and 4°C ($p=0.0015$, $p=0.03$).

Conclusions: TBR is increased with decreasing sample temperature, suggesting that the clinical exam of fluorescently labeled tissues may be improved at cooler temperatures. Both the rate of signal loss and the change in fluorescence with temperature observed for IRDye800CW are independent of the conjugating antibody.

8926-129, Session 4

Optical biopsy on head and neck tissue using full-field OCT: a pilot study

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Here we evaluate the clinical value of Full-Field OCT imaging in the management of patients with Head and Neck cancers by making a reliable histological diagnosis on FFOCT images produced during preoperative procedure. FFOCT performs a true “virtual extemporaneous exam” that we want to compare to the gold standard (extemporaneous and conventional histology with H&E staining). This new optical technology could be useful when diagnosing a lesion, cancerous or precancerous, or at the time of its surgical management.

Full-Field Optical Coherence Tomography virtually slices the tissue using white light interferometry to produce in-depth 2D images with an isotropic resolution around 1 micrometer. With such a high resolution FFOCT systems produce ”optical biopsy” images that are similar to that obtained with classical histology procedures, but without any staining and in only a few minutes.

We imaged tens of freshly excised samples from patients in the operating room, of mouth, tongue, epiglottis and larynx tissues, both healthy and cancerous. FFOCT images were acquired and later compared with histology of the same samples. Common features were identified and characteristics of each tissue type were matched in order to form an image atlas for pathologist training. We were able to identify indicators of tumors at the cellular scale such as heterogeneities in cell distribution, surrounding stroma, and anomalous keratinization.

In conclusion, FFOCT is a fast, non invasive, non destructive imaging tool that can be inserted into the pathology lab workflow and can provide a quick assessment of microscopic tissue architecture and content. Furthermore we are developing a similar system with a rigid endoscopic probe in order to do in vivo and in situ high-resolution imaging. Our probe could thus guide the surgeon in real time before and during excision and ensure a more precise gesture.

8926-130, Session 4

Widefield fluorescence imaging as an auxiliary tool to select the biopsy site for actinic cheilitis diagnosis

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Actinic cheilitis (AC) is considered a potentially malignant disorder that mainly affects the lower lip, and it is caused by prolonged sun exposure. Clinical diagnosis relies on visual inspection by a trained clinician, when suspected of dysplasia changes, a biopsy is required. The heteregenous characteristics of the AC, makes the choice of the biopsy site a difficult task. Fluorescence detection has been presented as a useful tool to to detect biochemical and morphological tissue features related to cancer diagnosis, but still its effectiveness to discriminate premalignant lesion is not completely defined. In this clinical study, 57 AC patients were investigated using widefield fluorescence imaging (WFI) to evaluate the efficacy of this technique as an auxiliary tool to biopsy site location. A handheld fluorescence system based on 400-450 nm LED illumination Distinct trained clinicians evaluate the patient either with the conventional examination or the WFI, and were blinded to the other evaluation. A biopsy site was chosen based on the clinical examination, and another site was chosen using the fluorescence visualization. A total of 114 punch biopsies were performed, and 93% of the tissue samples presented epithelial dysplasia. The majority of the sites that presented moderate or severe dysplasia were sites chosen by WFI, showing its efficiency to improve the diagnosis of AC.

Financial support provided by CNPq (475322/2011-8), CEPID-CEPOF/FAPESP (1998/14270-8).

8926-131, Session 4

Multimodal optical imaging approach for in? vivo diagnosis of oral cancer

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It is known that both biochemical and morphological changes accompany the transition from normal to neoplastic tissue. Yet most optical techniques investigated so far as potential screening and diagnostic tools for cancer have largely concentrated on detecting one or the other. We have recently developed a multimodal imaging system incorporating optical coherence tomography (OCT) and fluorescence lifetime imaging (FLIM) to simultaneously interrogate both set of biomarkers. For this study, we have used Syrian hamster cheek pouch model of epithelial cancer. A total of 53 OCT/FLIM images (FOV = 2x2 mm2) were acquired in-vivo from the cheek-pouch of hamster treated with the carcinogen B[a]P. Based on histopathology, 27 images were diagnosed as benign, 11 as either hyperplasia with dysplasia or carcinoma in situ (pre-cancerous), and 15 as squamous cell carcinoma. FLIM derived biochemical (e.g. normalized fluorescence intensity and lifetime values at three emission bands) and OCT derived morphological features (e.g. run length based texture features and several other features to characterize tissue architecture in terms of the number and nature of the layered structure of tissue) were estimated from each image. The FLIM features alone, the OCT features alone or a combination of both FLIM and OCT features were used to train statistical classifiers designed to discriminate benign, pre-cancerous and cancerous classes. The performance of each classifier was finally estimated via cross-validation.

8926-132, Session 4

Diagnosing indeterminate thyroid nodules with ESS: in vivo measurement improves diagnostic value of fine-needle aspiration biopsy

Jennifer E. Rosen, Nicholas J. Giordano, Ousama M. A’Amar, Eladio Rodriguez-Diaz, Irving J. Bigio, Stephanie L. Lee, Boston Univ. (United States)

Background: Thyroid nodules are common. The current gold standard, fine-needle aspiration biopsy (FNAB), yields 10-25% indeterminate results necessitating thyroidectomy for diagnosis. Elastic scattering spectroscopy (ESS) is a minimally invasive optical-biopsy technique, mediated by a fiberoptic probe that can fit through a 23 gauge biopsy needle. We hypothesized that combining a real time diagnostic test with high sensitivity (FNAB) to one with a high specificity (ESS) might increase the clinical utility of a gold standard.

Methods: We built a miniaturized ESS-integrated biopsy syringe that can fit through a 23-gauge biopsy needle and assessed the potential of ESS to pre-operatively differentiate benign from malignant thyroid nodules. An IRB approved protocol was conducted on patients undergoing ultrasound-guided FNAB of thyroid nodules. Approximately 15 ESS

spectra were collected per patient. Cells and ESS data were collected from within the thyroid nodule. Post-surgical pathology was our gold standard for indeterminate cytology.

Results: 108 patients enrolled in the study, of which 23 had indeterminate cytology. A waveform signature could discriminate benign from malignant disease. Preliminary results show a sensitivity of 0.92 and a specificity of 0.85.

Conclusion: ESS can reduce the number of indeterminate nodules that require surgical treatment. Preliminary analysis reveals a unique waveform signature that can differentiate benign vs. malignant thyroid nodules to improve targeting of biopsies. With the collection of further data, an algorithm using cytology and ESS data could potentially be used as an in-situ real time minimally invasive adjunct to conventional FNA cytology to improve diagnosis and prevent unnecessary surgery.

8926-133, Session 4

Combination of multimodal nonlinear microscopy and conventional histopathology for the assessment of head and neck squamous cell carcinoma

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Histopathologic examination is the current golden standard for diagnosing head and neck cancers. While staining histopathology relies on investigation of tissue biopsies, which are labelled by staining agents or immunohistochemical markers, multimodal nonlinear microscopy enables label-free examination of head and neck tissue sections and is as such potentially applicable to analyze tissue in vivo in depths up to 1 mm. However, so far investigations on the correlation between morphologic disease markers, e.g., architectural and cytological changes during carcinogenesis, and molecular markers, which are accessible by nonlinear imaging, have not been performed. Hence, in this work, histopathology using a comprehensive set of multiple staining agents and immunohistochemical markers was compared to multimodal nonlinear imaging combining second harmonic generation (SHG), two photon excited fluorescence (TPEF) and coherent anti-Stokes Raman scattering (CARS) for visualizing characteristic morphologic structures and the chemical composition of the tissue. This study being performed on samples from 10 patients illustrates the prospects of multimodal nonlinear microscopy for intraoperative imaging in head and neck surgery and investigates characteristics, which are not accessible by multimodal microscopy. Implementation of multimodal nonlinear imaging into surgical microscopes will improve the accuracy for resection of tumors by improved visualization of tissue pathologies with subcellular resolution in comparison to alternative imaging modalities.

Acknowledgement

Financial support from European Union via the ‘Europäischer Fonds für Regionale Entwicklung (EFRE)’ and the ‘Thüringer Ministerium für Bildung Wissenschaft und Kultur (TMBWK)’ (projects: B578-06001, 14.90 HWP and B714-07037) and the German Federal Ministry for Science and Education (BMBF) MediCARS (FKZ: 13N10774) is gratefully acknowledged.

8926-134, Session 4

Reducing insufficiency in thyroid biopsies: improving the diagnostic value of a gold standard, an integrated spectroscopy syringe

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Introduction. Thyroid nodules are common. The current standard of diagnosis, ultrasound guided fine needle aspiration biopsy (FNAB) yields insufficient results between 5 – 10% of the time, necessitating a repeat biopsy or surgery for diagnosis. Elastic scattering spectroscopy (ESS) is a minimally invasive optical-biopsy technique that we have adapted for use in the clinic by miniaturizing it to fit through a 23 gauge needle. We hypothesized that ESS in vivo can reduce our insufficiency rate and improve the diagnostic value of a gold standard.

Methods. Under an IRB approved protocol, patients receiving a FNAB had ESS data collected using our ESS integrated biopsy probe, and when applicable, the probe was guided towards solid components of a nodule for cell collection. Up to 5 subsequent and separate passes with a standard 23 gauge needle were taken. Insufficiency rates and specimen adequacy were compared between needle passes.

Results. 134 patients were enrolled and had data available for analysis. Only 2.2% had an insufficient biopsy when performing the biopsy with the ESS probe, compared to 10% with a standard needle.

Conclusion. A clinical trial using an integrated ESS/biopsy probe in vivo is feasible and acceptable to patients. Both spectral data and cytologic material are adequate in the majority of patients. The real-time feedback provided by ESS may significantly increase the likelihood of material adequacy. With further accrual and analysis, this ESS device may establish standardized criteria for adequate FNAB specimens.

8926-135, Session 5

Determination of tissue optical properties in ALA-mediated Head and Neck PDT treated patients

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Determination of optical properties (absorption (?a) and scattering (?s’) coefficients) in human tissue is important when it comes to accurate calculation of fluence rate in and around tissue area. ALA application to the tissue induces production of protoporphyrin IX when activated by red light. Changes in the tissue optical properties can send information such as treatment outcome and tissue drug concentration.

Patients in this study were treated with PDT for head and neck mucosal dysplasia. They were enrolled in a phase I study of escalating light doses and oral ALA with 60mg/kg. Red light at 630nm was administered to the tumor from a laser. The light dose was escalated from 50-200J/cm2 with a measured fluence rate at tissue surface of 100mW/cm2.

We developed a light detection device for the purpose of determining optical properties in vivo using the semi-infinite method. The light detection device consists of two parallel, placed 5mm apart. In one of the catheters a 2 mm long linear diffusing light source is placed while in the second catheter, a calibrated isotropic detector is placed. The detector is scanned along the length of the light source containing catheter. Scans are done with the device placed on the treatment area (tumor) and on the normal tissue. Optical properties were measured in-vivo before and after PDT delivery for both normal tissue and tumor.

8926-136, Session 5

Plasmonic nanobubble theranostics for intra-operative and preventive treatment of head and neck squamous cell carcinoma

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Chemoradiation-resistant cancer cells and unresectable micro-tumors limit treatment efficacy and lead to high non-specific toxicity or recurrence in head and neck cancers. We show the cancer cell-specific, on-demand enhancement of the chemo- and chemoradiation therapy with mechanical intracellular impact of plasmonic nanobubbles, a laser pulse-induced explosive nano-event, not a particle. We report cellular mechanisms of cancer cell-specific detection and enhancement of the entry drug and X-ray dose and validate these mechanisms in vitro and in vivo for head and neck squamous cell carcinoma. Plasmonic nanobubble technology showed more than 10-fold enhancement of the therapeutic efficacy compared to standard chemoradiation in murine models of primary, microscopic residual and recurrent diseases. At the same time our technology efficiently spared adjacent normal tissues due to the reduction of the effective therapeutic doses of drug by 30-40 fold, X-rays by 15-fold and the treatment time to a single procedure. The developed plasmonic nanobubble technology transforms a standard macro-therapy into a cell-level on-demand theranostic treatment for primary, adjuvant and adjunct applications.

8926-137, Session 6

Investigation on laser induced salivary stone fragmentation

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In-vitro investigation of photon-based techniques to identify the composition of salivary stones and of the fragmentation of salivary stones by means of Ho:YAG-laser.

Salivary stones extracted from patients with clinical symptoms of sialothiasis were examined. After measuring sizes and volumes DE CT scans were performed to classify the stones by their density. Fluorescence measurements were performed either by taking images under blue light excitation light as well as measuring excitation-emission-matrixes. Fragmentation was performed in an aquarium set-up equipped with a mesh of 1mm holes, using a Ho:YAG-laser to deliver defined laser pulses to the stone surface. From these data fragmentation rates were calculated. Raman and FTIR-spectroscopy were used to identify the composition of the stone.

Blue light fluorescence excitation resulted in either fluorescence in the green spectral region or in a combination of green and red fluorescence emission, proven by EEM-measurement. Raman spectroscopy showed a mixture of carbonate apatite and keratin. Salivary stones could be differentiated by means of density measurements performed using DECT techniques. A correlation with FTIR-spectroscopy results is still under investigation.

Fragmentation experiments resulted in a dependency to the applied energy/pulse if the evaluation implies the ratio of fragmented weight to pulse, while the ratio fragmented weight to energy remains about constant for the three laser parameter used. With respect to the clinical application it must be kept in mind that Ho:YAG laser energy also have an impact on the surrounding tissue, thus lowest energy/pulse should be used.

Using photonic techniques salivary stones could be distinguished by their composition. Clinical treatment with Ho:YAG-laser at low pulse energy prevent for side effects.

8926-138, Session 6

Accuracy of optical navigation systems for automatic head surgery: optical tracking versus optical coherence tomography

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In recent years, optical coherence tomography (OCT) has gained increasing interest not only as an imaging system in medicine but also as a measuring device in technical and life science applications. In this paper, OCT is used as a navigation system in a simulated image guided surgery (IGS) and compared, in terms of accuracy, to state of the art triangulation-based optical tracking (OT). The simulation comprises the realization of a single-channel for a cochlear implant (CI) surgery. For this purpose, an experimental setup with a combined OCT and cutting laser, and an external OT is used. The simulated robot assisted intervention in hard tissue includes calibration, planning, navigation, processing, and evaluation. The system's components not only have to be calibrated themselves, but also registered with respect to the other system's components. For navigation and evaluation purposes, we equipped the sample with passive and spherical markers of image device dependent materials and diameters. The evaluation is carried out determining the error between actual and target ablation, and comparing the results of the two different navigation technologies. First results show that OCT is, in contrast to OT, suitable for high accuracy interventions such as CI surgery, fulfilling its accuracy demands. Thus, this lends preliminary support to the assumption that OCT may be used as an external high-accuracy guidance system.

8926-140, Session 6

New photodynamic therapy dosimetry model for head and neck cancer *(Invited Paper)*

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Photodynamic therapy (PDT) has shown promise in the treatment of patients with early stage head and neck cancer. Additionally, several studies demonstrated that interstitial PDT (iPDT) is effective for patients with locally advanced head and neck cancer. However, there is need for a predictive dosimetry model that can assist physicians to improve the administration of PDT and iPDT.

We have developed a new PDT dosimetry model that includes hardware and a biomarker that can be used to monitor the therapy in real time and evaluate the deposited PDT dose and potential tumor response within 48 hrs. The model can be applied to PDT and iPDT with any photosensitizer and light source within the range of 400-830 nm. In this talk we will present the model and initial results from a clinical study.

Acknowledgements: This work was supported, in part, by NIH/NCI Grants PO1CA055791 and (716) 829-2593.

8926-141, Session 6

Efficient tissue ablation using a laser tunable in the water absorption band at 3 microns with little collateral damage

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Lasers have the possibility for significantly advancing medical diagnostics and treatment. At high power, they are typically used as cutting tools during surgery. For lasers that are used as knifes, radiation wavelengths in the far ultraviolet around 190 nm and in the near infrared spectral regions are favored because tissue has high contents of collagen and water. Collagen has an absorption peak around 190 nm, water in the near infrared around 3000 nm. Changing the wavelength across the absorption peak will result in significant differences in laser tissue interactions. Tunable lasers in the infrared that could optimize the laser tissue interaction for ablation and/or coagulation are not available until now besides the Free Electron Laser (FEL). Here we demonstrate efficient tissue ablation using a table-top mid-IR laser tunable between 3,000 to 3,500 nm. A detailed study of the ablation has been conducted in different tissues and little collateral thermal damage has been found at a distance above 10-20 microns from the ablated surface. Furthermore, no signs for mechanical damage could be see in conventional histology and by examining birefringent activity of the samples using a pair of cross polarizing filters.

8926-142, Session 7

Optical screening in upper aerodigestive tract (pre)malignancies: an overview

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Optical screening methods are meant to enhance the detection and delimitation of superficial mucosal lesions in vivo. Even though large, randomised trials are missing, different methods such as autofluorescence imaging and narrow band imaging have yielded promising results and may become routine adjunct diagnostic tools in the near future.

OCIS codes: Endoscopic imaging (170.2150), Optical diagnostics for medicine (170.4580)

1. Objective

Optical screening methods have recently drawn considerable attention, as they seem to be helpful with both the detection and the delimitation of superficial mucosal lesions. Various methods such as fluorescence-based techniques (i.e. autofluorescence imaging and enhanced fluorescence imaging (various systems and distributors)) as well as techniques mainly focusing on contrast enhancement (e.g. narrow band imaging (Olympus Medical Systems GmbH) or SPIES (KARL STORZ GmbH)) have been put forward recently and are marketed for the upper aerodigestive tract (UADT). Our intent is to provide an up-to-date review of the literature in the setting of head and neck pathologies as well as describing own results and areas of future research.

2. Materials and Methods

The relevant literature (mostly PubMed) was screened for clinical studies on the application of optical screening methods to aid the diagnosis in UADT premalignancies and early cancers. Personal experience was taken into consideration as well.

3. Results and Discussion

Even though optical screening has not been investigated in randomized trials large enough so as to give an evidence-based recommendation to and when to use it, evidence is mounting that it might be a useful adjunct diagnostic tool in addition to regular, high-definition endoscopy. Especially autofluorescence imaging and narrow band imaging have been quite thoroughly investigated, and both seem to enhance the sensitivity of pre-/early cancer detection within the UADT. At the same time, all methods presented are quite low on specificity, emphasizing the need for additional, non-invasive optical techniques to further differentiate the mucosal lesions detected by optical screening.

4. Conclusion

Optical screening methods such as autofluorescence imaging and narrow band imaging have shown to be able to increase the sensitivity of pre- and early cancerous mucosal lesions of the UADT if used in conjunction with regular endoscopy in smaller trials, but real scientific evidence is still missing and the true value of these methods are yet to be determined.

8926-143, Session 7

Digital 3-D modeling of the pediatric subglottis based on optical coherence tomography: a preliminary investigation *(Invited Paper)*

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BACKGROUND: The subglottis forms the narrowest portion of the pediatric airway, consisting of the circumferential cricoid ring and delicate mucosa. In pediatric patients under long-term endotracheal intubation, the subglottis is predisposed to epithelial injury which, in rare cases, may lead to fibrosis and subglottic stenosis. Optical coherence tomography (OCT) is an imaging modality based on the principles of low coherence interferometry. OCT measures back-scattering of light from sample tissue based on differences in tissue optical properties. The resulting reflectivity profile is used to generate cross-sectional images of tissue with micrometer resolution and millimeter depth. We investigate the processing of OCT data to render digital three-dimensional (3-D) models of the pediatric subglottis.

METHODS: OCT was performed on 44 pediatric patients (age 2-16 years) undergoing minor head and neck surgery and 12 intubated neonates (ages 2 - 175 days) requiring ventilatory support. A frequency domain OCT system with a 0.7 mm rotary optical probe was used to image the trachea, subglottis, glottis and supraglottis from within the endotracheal tube. Beginning in the proximal trachea, the probe was rotated at 25 Hz and retracted by an automated stage at 6.25 mm/sec (250 ?m separation between consecutive frames). OCT data was processed in MATLAB software, including image rescaling, anterior-posterior alignment and noise removal. Post-processing data was used to construct 3-D airway models in Amira software.

RESULTS: Subglottic airway landmarks were clearly delineated on OCT images from 41 (73%) airways, including identification of microanatomy (epithelium, basement membrane, lamina propria, glandular structures) and cartilage. Digital 3-D models allowed for user-controlled scrolling through the airway in axial, sagittal or coronal views. 3-D Models clearly depicted the layered microstructure of the subglottis in multiple orientations and allowed for direct comparison of subepithelial structure with adjacent airway levels.

CONCLUSION: OCT-based digital 3-D models allow for visualization of the layered subepithelial structure of the subglottis in multiple orientations. This may provide clinicians with invaluable information about early, subepithelial changes in the subglottic mucosae caused from prolonged intubation.

8926-144, Session 7

Shrinkage of porcine cutaneous specimen after formalin fixation and histopathology preparation: utilising OCT for dimensional change measurements

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A porcine model was utilized in this study in order to evaluate and measure the percentages of both horizontal and vertical dimensional changes (contraction or shrinkage and expansion), using a new in-situ OCT oral instrument as well as the standard OCT dermatology instrument, before formalin fixation (fresh or dry porcine skin), after seven days fixation of the porcine model in 10% NBF (Neutral Buffered Formalin), as fixation for a short period of time (between 24-48 hours) lead to incomplete processing, thus the duration of fixation can affect the degree of tissue shrinkage. In addition, we have also measured the dimensional changes for the porcine skin histopathology slides.

Results show that the mean percentage of tissue expansion and shrinkage in horizontal (X) plane as a result of formalin fixation was about 7.22% for OCT skin instrument, while for OCT oral system was about 7.40%, whereas in vertical (Z) plane the mean percentage of tissue shrinkage was 7.01% and 7.44% for OCT skin and oral systems respectively. While the mean percentage of tissue expansion and shrinkage in horizontal (X) plane for the histopathology sections/images was greater compared to formalin fixation of the porcine skin tissue and was about 11.33% for OCT skin instrument, while for OCT oral system was about 11.23%, whereas in vertical (Z) plane the mean percentage of tissue shrinkage was 12.45% and 12.82% for OCT skin and oral systems respectively.

8926-145, Session 7

Transitioning long-range optical coherence tomography of the pediatric upper airway from the operating room to the clinic

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Upper airway (UA) obstruction affects up to 3% of children. Adenotonsillectomy (AT) significantly relieves symptomatic UA obstruction in the majority of children; however, there are still many who do not improve. The decision to proceed to surgery is based on clinical impressions, most often without quantitative anatomic criteria. Long-range optical coherence tomography (LR-OCT) is an optical imaging modality that utilizes infrared light to obtain high-resolution cross-sectional images. LR-OCT can be used as a means to image UA anatomy in children to precisely identify loci of obstruction. However, there are challenges to transitioning from imaging in the operating room to imaging awake children. The material of the sheath is important to generating high-quality images while minimizing patient discomfort.

8926-146, Session 7

Evaluation of optical coherence tomography and probe-based confocal laser endomicroscopy to discriminate lesions of the upper aerodigestive tract

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OCT helps to discriminate invasive from non-invasive lesions in the UADT; pCLE might be helpful to distinguish non-invasive lesions. Results of a clinical feasibility trial applying both OCT and pCLE in conjunction are presented.

OCIS codes: Confocal microscopy (180.1790), Optical Coherence Tomography (170.4500)

1. Objective

Gold standard in the evaluation of lesions of the upper aerodigestive tract (UADT) is white light examination followed by invasive tissue biopsy, which is cost-intensive and time-consuming. As previously shown, Optical Coherence Tomography (OCT), can differentiate between invasive and non-invasive lesions. In this prospective clinical trial, we evaluated the potential of probe-based Confocal Laser Endomicroscopy (pCLE), a high-resolution optical imaging modality, for the in-vivo differentiation of various non-invasive lesions.

2. Materials and Methods

OCT-examinations were performed in 23 patients with 35 primary leukoplakias or erythroplakias of the UADT. Lesions showing a normal tissue lining but increased epithelial thickness (n=30), which are presumably indistinguishable with OCT, were further investigated with pCLE using intravenous Fluorescein as a contrast agent. The suspected diagnosis was compared to the histopathological result.

3. Results and Discussion

OCT served to distinguish between non-invasive and invasive disease as expected. pCLE provided further information on the tissue in subcellular resolution. In 2 out of 30 cases, low image quality prevented further classification. Of the remaining 28 lesions, 17/28 histopathological diagnoses (61%) were correctly predicted; 8 were interpreted as epithelial hyperplasia (6/8 true), one as low grade dysplasia (0/1 true), and 19 as moderate to high grade dysplasia (11/19 true). Moderate to high grade dysplasia was correctly suspected in 11/12 cases (92%), whilst hyperplasia was overrated as epithelial dysplasia in 8/16 cases (50%).

4. Conclusion

When used in conjunction with OCT, pCLE seems helpful for a further discrimination of various non-invasive lesions of the UADT, though the method tends to overrate the severity of the changes.

Acknowledgement: This investigation was supported by the device grant of the DFG (INST 409/88-1 FUGG).

8926-147, Session 7

Raman spectroscopy and oral exfoliative cytology

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Introduction Early detection of oral cancers can substantially improve disease-free survival rates. As ex vivo and in vivo Raman spectroscopic (RS) studies on oral cancer have demonstrated the applicability of RS in identifying not only malignant and premalignant conditions but also cancer-field-effects: the earliest events in oral carcinogenesis 1,2. RS has also been explored for cervical exfoliated cells analysis3. Exfoliated cells are associated with several advantages like non-invasive sampling, higher patient compliance, transportation and analysis at a central facility: obviating need for on-site instrumentation4,5. Thus, oral exfoliative cytology coupled with RS may serve as a useful adjunct for oral cancer screening.

Materials and Methods In this study, exfoliated cells from healthy controls with and without tobacco habits, premalignant lesions (leukoplakia, OSMF and tobacco-pouch-keratosis) and their contralateral mucosa were collected using a Cytobrush. Cells were harvested by vortexing and centrifugation at 6000 rpm. The cellular yield was ascertained using Neubauer's chamber. Cell pellets were placed on a CaF2 window and Raman spectra were acquired using a Raman microprobe (40X objective) coupled HE-785 Raman spectrometer. Approximately 7 spectra were recorded from each pellet, following which pellet was smeared onto a glass slide, fixed in 95% ethanol and subjected to Pap staining for cytological diagnosis (gold standard).

Results and Discussion Preliminary PC-LDA followed by leave-one-out cross validation indicate delineation of cells from healthy and all pathological conditions. A tendency of classification was also seen between cells from contralateral, healthy tobacco and site of premalignant lesions. These results will be validated by cytological findings, which will serve as the basis for building standard models of each condition.

8926-148, Session 7

Raman spectroscopy of oral tissues: correlation of spectral and biochemical markers

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Introduction

Optical spectroscopic methods are being explored as novel tools for early and non-invasive cancer diagnosis. Both ex vivo and in vivo Raman spectroscopic studies carried out in oral cancer over the past decade have demonstrated that, spectra of normal tissues are rich in lipids, tumor spectra show a predominance of proteins1-3. Therefore, in the present study, we have carried out Raman and biochemical studies on same tissues to correlate spectral markers and biochemical composition of normal and tumor oral tissues.

Materials and Methods

Spectra of 20 pairs of normal and tumor oral tissues were acquired using fiber-optic probe coupled HE-785 Raman spectrometer. Intensity associated with lipid (1440 cm-1) and protein (1450 and 1660 cm-1) bands were computed using curve-deconvolution method. Same tissues were then subjected to biochemical estimations of major biomolecules, i.e., protein, lipid and phospholipids.

Results and Discussion

The intensity of the lipid band was found to be higher in normal tissues with respect to tumors, and the protein band was higher in tumors compared to normal tissues. Biochemical estimation yielded similar results i.e. high protein to lipid or phospholipid ratio in tumors with-respect to normal tissues. These differences were found to be statistically significant.

Conclusion

Findings of curve-deconvolution and biochemical estimation correlate very well and corroborate the spectral profile noted in earlier studies.

8926-149, Session PSun

Probe-based confocal laser endomicroscopy in head and neck malignancies using tumor-specific contrast agents: early preclinical experience

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Abstract: Specimens from UADT SCCs were subjected to ex-vivo pCLE and CLSM following application of different, tumor-specific contrast agents. pCLE allowed for visualization of tissue structures at subcellular resolution, holding promise to become an adjunct diagnostic method.

OCIS codes: Confocal microscopy (180.1790), Fluorescence microscopy (180.2520)

1. Objective

Malignancies of the upper aerodigestive tract (UADT) are conventionally diagnosed by invasive tissue biopsy, which is time-consuming, costly and prone to errors. Probe-based Confocal Laser Endomicroscopy (pCLE) is a novel non-invasive technique which offers in vivo imaging with subcellular resolution. In this study we evaluated the potential use of pCLE in combination with non-specific (Fluorescein) and specific contrast agents (fluorescent EGFR- and EpCAM-antibodies) to distinguish between healthy mucosa and invasive carcinoma.

2. Materials and Methods

Tissue samples from healthy mucosa and squamous cell carcinoma of the UADT were taken during surgery. After topical application of either FITC-labeled EGFR-antibodies, FITC-labeled EpCAM-antibodies or FITC-controls, the samples were examined using different pCLE-probes (varying resolution, field-of-view and penetration in conjunction with the Mauna Kea CellVizio System (Mauna Kea Tech, Paris, France)), and a Confocal Laser Scanning Microscope (CLSM) (DM IRBE, Leica, Wetzlar, Germany). Images were then compared to the corresponding histological slides and cryosections, which were in parts stained with FICT-labeled EGFR- or EpCAM-antibody.

3. Results and Discussion

Initial results of n=20 examined cases showed that pCLE in combination with the used, tumor-specific markers shows tumor-cell specific staining, which generally corresponded to the results obtained from CLSM and histology, although the different orientation did not allow for direct comparison. Imaging of different layers with a varying resolution was possible using three distinct pCLE-probes. In addition, time and concentration series allowed for the determination of usable application parameters.

4. Conclusion

pCLE is a non-invasive method for high-resolution in-vivo tissue imaging. When appropriate fluorescent markers are used, the method has the potential to specifically highlight tumor cells and might therefore be helpful for diagnosis as well as tumor resection.

Acknowledgement: This investigation was supported by the device grant of the DFG (INST 409/88-1 FUGG).

Conference 8926D: Diagnostic and Therapeutic Applications of Light in Cardiology

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8926 Photonic Therapeutics and Diagnostics X

8926-66, Session 13

Optical methods to analyze blood clotting *(Invited Paper)*

Seemantini K. Nadkarni, Harvard Medical School (United States)

No Abstract Available

8926-67, Session 13

Intravascular NIRS *(Invited Paper)*

James Goldstein, Beaumont Hospital (United States)

No Abstract Available

8926-68, Session 13

TBD *(Invited Paper)*

Jeffrey Southard, Univ. of California, Davis (United States)

No Abstract Available

8926-69, Session 14

Performance improvement by a broadband super-luminescent diode light source in 1.7-µm spectroscopic spectral-domain optical coherence tomography for lipid distribution imaging in a coronary artery

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We improve performance by a super-luminescent diode (SLD) light source in 1.7-?m spectral-domain optical coherence tomography (SD-OCT) system for lipid distribution imaging in coronary arteries. As the light source, three customized SLDs are combined in parallel polarization by standard single mode couplers. The center wavelengths of the SLDs are 1650, 1685 and 1735nm, which are selected for broad spectrum with bandwidth of 115nm over the spectrometer band. In the previous report, the 1.7-?m SD-OCT system with a super-continuum light source has the sensitivity of 108dB with slow A-scan rate (0.96kHz) and high emission power (74mW). On the contrary, the 1.7-?m SLD-based SD-OCT system reaches the sensitivity over 100dB because of lower noise light source in spite of increasing the A-scan rate to 47kHz and decreasing the emission power to 8.4mW. The system performance is applicable to real-time observation in coronary arteries.

For demonstration with the 1.7-?m SLD-based SD-OCT system, we use a carotid artery with a nylon tube injected with lipid as an in-vitro artery model and a prototype of OCT catheter designed for 1.7-?m. The in-vitro model is measured by pulling back the catheter with the B-scan rate of 94Hz and the pullback rate of 20mm/s. The spectroscopic OCT algorism by absorption characteristics of lipid is applied to the measured fringes and lipid distribution is visualized from lipid contents calculated by pixel. We prove that the sensitivity and specificity between artery and lipid areas in the in-vitro model are more than 90% at the optimal threshold.

8926-71, Session 14

Application of autofluorescence lifetime metrology as a label-free technique to assess heart disease

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Fluorescence lifetime measurements of endogenous fluorophores such as NADH and flavoproteins present an opportunity to discern functional information concerning the energetics of the heart without the inherent issues of toxicity or systemic effects involved with the introduction of exogenous compounds. In addition, autofluorescence from molecules such as collagen may provide information on structural changes to the heart. Measurements of fluorescence lifetime are independent of fluorophore concentration, excitation intensity, sample attenuation and other experimental artefacts and can also report on changes to the fluorophore microenvironment, e.g. pH and protein-binding. Thus we are interested to develop autofluorescence lifetime (AFL) based techniques for myocardial 'optical biopsy'. Here we report the application of a custom fibre-optic probe-based time-resolved spectrofluorometer utilizing time-correlated single photon counting (TCSPC) that we developed to characterise the autofluorescence signatures associated with histological, morphological, metabolic and functional changes in myocardial tissue in health and disease states. Following myocardial infarction, sections of myocardium are replaced by scar and the remaining myocardium undergoes remodelling. We studied an in vivo rat left anterior descending coronary artery ligation model 16 weeks post-infarction. We observed no significant difference in the AFL signals between the age-matched control (AMC) hearts in different anatomical locations but did observe a significant difference in AFL between the infarcted zone in infarcted hearts compared to AMC ($P<0.0001$). There was also a significant difference between the remaining viable myocardium of the posterior wall (which demonstrates hypertrophic remodelling in this model) from AMC ($p<0.01$). This technique could be readily translatable into cardiology practice.

8926-72, Session 14

Quantitative evaluation of atherosclerotic plaque phantom by near-infrared multispectral imaging with three wavelengths

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Atherosclerosis is a primary cause of critical ischemic disease. The risk of critical event is involved the content of lipid in unstable plaque. Near-infrared (NIR) range is effective for diagnosis of atherosclerotic plaque because of the absorption peaks of lipid. NIR multispectral imaging (NIR-MSI) is suitable for the evaluation of plaque because it can provide spectroscopic information and spatial image quickly with a simple measurement system. The purposes of this study were to determine the optimal wavelengths for observing atherosclerotic plaque and to evaluate the lipid concentration in plaque phantoms quantitatively with a NIR-MSI system. The NIR-MSI system was constructed with a supercontinuum light, a grating spectrometer and MCT camera. Plaque phantoms with different concentration of lipid were prepared by mixing the bovine fat and biological soft tissue model to mimic the different stages of unstable plaque. We evaluated the phantoms by the NIR-MSI system with three wavelengths in the band at 1200 and 1700 nm. Multispectral images were processed by spectral angle mapper method. As a result,

the lipid areas of phantoms were effectively highlighted by using three wavelengths to reproduce the shape of absorption peak of lipid, one is the top of the peak (1210 or 1730 nm) and others are the bottoms of peak sandwiching the peak. In addition, the concentrations of lipid areas were classified according to the similarity between a measured spectrum and a reference spectrum. These results suggested the possibility of image enhancement and quantitative evaluation of lipid in unstable plaque with NIR-MSI.

8926-73, Session 15

Calcium and voltage imaging in arrhythmia models by high-speed microscopy

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Alterations in intracellular cardiomyocyte calcium handling have a key role in initiating and sustaining arrhythmias. Arrhythmogenic calcium leak from sarcoplasmic reticulum (SR) can be attributed to all means by which calcium exits the SR store in an abnormal fashion. Abnormal SR calcium exit maymanifest as intracellular Ca2+ sparks and/or Ca2+ waves.

Ca2+ signaling in arrhythmogenesis has been mainly studied in isolated cardiomyocytes and given that the extracellular matrix influences both Ca2+ and membrane potential dynamics in the intact heart and underlies environmentally mediated changes, understanding how Ca2+ and voltage are regulated in the intact heart will represent a tremendous advancement in the understanding of arrhythmogenic mechanisms. Using novel high-speed multiphoton microscopy techinques, such as multispot and random access, we investigated animal models with inherited and acquired arrhythmias to assess the role of Ca2+ and voltage signals as arrhythmia triggers in cell and subcellular components of the intact heart and correlate these with electrophysiology.

8926-74, Session 15

Prediction of myocardial damage depth induced by extracellular photosensitization reaction using fluorescence measurement in vivo

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We experimentally studied the correlation between myocardial damage depth due to the extracellular photosensitization reaction (PR) using talaporfin sodium and fluorescence-fall amount (FA), which is calculated from the measured backscattering fluorescence intensity via a manipulatable 7 Fr. laser catheter during the PR operation in vivo to establish treatment depth predictor for a non-thermal tachyarrhythmia treatment. The PR was performed to left and/or right ventricle in the open-chest canine heart. The laser irradiation of 663±2 nm in wavelength via the laser catheter was operated 15 min after the intravenous administration of talaporfin sodium with concentration of 20-40 ?g/ml in plasma. The irradiation was operated with irradiance of 5, 10, 20 W/cm2, and duration of 5, 10, 20 s. Backscattering fluorescence of 710±2 nm in wavelength was measured via the laser catheter during the PR. The FA was calculated multiplying the irradiation duration by the fluorescence-fall, which is subtraction of the fluorescence intensity at the kickoff and end of the irradiation. The canine heart was extracted 1 week after the PR and HE stained specimen was histologically evaluated. The correlation of the myocardial damage depth and FA was investigated. We found that FA obtained a logarithmic relation to the myocardial damage depth, and also FA was affected by the contact force of the laser catheter tip to the myocardium. We think that the FA might be available to predict the PR induced myocardial damage depth for the application of tachyarrhythmia treatment under catheterization in vivo.

8926-75, Session 15

Three-dimensional quantification of myofiber orientation and tractography using optical coherence tomography

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Changes in orientations of myofibers are associated with diseases such as arrhythmia, abnormal contraction, and cardiomyopathy. We present a method of quantifying 3D fiber orientation using optical coherence tomography (OCT). We determined the orientation within en face images and back projected the fiber into 3D space. Next, we formulated the fiber tracking problem as Markov state model and utilize particle fiber techniques to reconstruct tractography. The predications of paths were based on prior probability distribution of orientations. The estimation of tract was calculated as the weighted mean of particles in each step.

Three-dimensional image sets were acquired from swine hearts (n=5) with a Thorlabs Telesto OCT system. We validated our algorithm by processing our algorithm on samples rotated in either azimuth (test1, ??1'=90º, ??1'=0º) or polar angle (test2, ??2'=90º, ??2'=0º). In each experiment, we observed exact change in rotated angle (??1=89.14º±7.11º, ??2=13.99º ± 3.01º) and slight change in the other angle (??1=1.76º±1.35º, ??2=4.96º±4.02º). We also compared the results from our algorithm with those from manually measurements. The two compared well with a slope of 0.9762 (R2=0.7506) and 0.99 (R2=0.8091) in the atria and ventricle, respectively. The results demonstrate that our algorithm is able to provide accurate orientations of myofibers. We perform fiber tractography in three-dimensional space and the reconstructed fiber tracts match the streamline of fibers in OCT images.

The experimental results demonstrate the feasibility of extracting three-dimensional information of myofibers. Our method paves a way for further in vivo experiments and may help to improve diagnosis and clinical monitoring.

8926-76, Session 15

Is it possible to prevent morbidity on post cardiovascular surgery applying low level laser therapy?

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Background and Objective: Complications following cardiovascular surgery incision are common, a 47% mortality rate remaining. Low Level Laser Therapy (LLLT) has been employed mainly to its effectiveness analgesic and anti-inflammatory actions, aiding the tissue repair process. The study's aim was to evaluate infrared LLLT onto surgical incision in patients submitted to cardiovascular surgery. Methods: 40 patients were divided in two groups: Placebo Group (G1) – conventional therapy + "Laser pointer" and Laser Group (G2) – conventional therapy + Infrared Laser irradiation on surgical incision. C.W. Diode Laser surround the surgical incision, on immediate Post Operative (PO), 1st PO and 3rd PO with the following parameters was employed: ?=830nm, P=35mW. Results: G2 didn't present any morbidity although in G1 5 patients developed incision dehiscence and infection. On 7thPO, still a large

amount of G1 patients referred pain and unquestionable inflammatory signs surrounding the surgical wound, when compared to G2. Besides, hospital stay in Laser Group was twice shorter than in Placebo (p-value=0.001). Conclusion: Infrared wavelength's Lasertherapy denoted to be safe and exceptionally valuable tools in preventing morbidities on post cardiovascular surgeries.

8926-77, Session 15

Study's significance from arterial elasticity and variation in arterial blood pressure for normotensive and hypertensive patients applying pre and post lasertherapy: preliminary results

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Background/Objective: Systemic Arterial Hypertension (SAH) is a multifactorial disease that affects 600 million people worldwide and accounts over 50 million of Brazilian population. The purpose was to evaluate the impact of low level laser therapy (LLLT) effect in the individual's blood pressure. Methods: 8 individuals with normal and high blood pressure were measured onto the radial artery waveform by HDI/PulseWave™ Sensor CR-2000 Profiling System analyzed: systolic and diastolic blood pressure (SBP and DBP, respectively), systemic blood pressure (SBP), mean arterial pressure (MAP), pulse pressure (PP), heart rate (HR), estimated cardiac output (CO), arterial elasticity index (AEI) and systemic vascular resistance (SVR), were assessed. C.W. Diode Laser (?-780nm, Power= 50mW, Time= 30s, Fluency= 50J/cm2, Energy/point= 1.5J) for 6 times were applied into the mouth. Results: A non-invasive LLLT approach, measured by HDI/PulseWave profiler unit for hypertensive group and could drop the numbers of all cardiovascular parameters, such as: SBP=147±15 mmHg vs 140±18 mmHg, SVR=1641±143 vs 1552±143 dynes.s.cm-5, TVI=142±25 vs 118±26 dynes.s.cm-5, AEI=14±4 vs 16±5 mL/mmHgx10, median arterial pressure-MAP=3±1 vs 5±2 mL/mmHgx100) while it was compared w/ normotensive group: (SBP=118±11 vs 117±13 mmHg, SVR=1209±333 vs 1197±356 dynes.s.cm-5, TVI=108±43 vs 122±55 dynes.s.cm-5, AEI=22±11 vs 19±6 mL/mmHgx10, MAP=8±4 vs 8±4 mL/mHgx100). Conclusion: Pre and post LLLT preliminary data showed substantial improvements w/ hemodynamic trends for hypertension patients. Thus, Lasertherapy seems to be an effective tool providing better arterial elasticity and it's a safe treatment to diminish the high blood pressure without side-effect, then reducing the cardiovascular risk for hypertensive patients.

8926-78, Session 16

Fiber-based combined OCT and two-photon luminescence imaging system for detection of thin-cap fibroatheroma

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Atherosclerosis and plaque rupture leading to heart attack and stroke remain the leading cause of death worldwide. Plaque-based macrophages are an important early cellular marker that indicates plaque vulnerability. In vivo intravascular macrophage detection in the context of plaque structures with high spatial resolution, high sensitivity and specificity is potentially of great clinical significance. We present use of gold nanorods, providing superior two-photon luminescence (TPL) brightness, as a contrast agent to target macrophages in thin-cap fibroatheromas (TCFA) for TPL imaging. A combined optical coherence tomography (OCT)-TPL imaging system using a photonic crystal fiber (PCF) was used to detect nanorod-loaded macrophages in TCFA samples. The OCT-TPL imaging system incorporates swept-source OCT (1310 nm) and TPL imaging (at 760-1040 nm excitation). A PCF was used to simultaneously transmit single-mode OCT/TPL excitation/emission light to/from the sample. Preliminary results show that PCF-based OCT-TPL imaging system can detect 2-D TPL emissions from a phantom sample with co-localized 3-D OCT signals. The merged OCT-TPL images simultaneously depict the sample surface structure and composition. TCFA samples (injected with nanorods) are used to verify the capability of the OCT-TPL system. Our results suggest that PCF-based combined OCT-TPL imaging is a promising technique to detect macrophages and plaque structures simultaneously in TCFAs.

8926-79, Session 16

Multi-functional intravascular imaging system for vulnerable plaque diagnosis

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In order to diagnose high risk vulnerable plaques at earlier stage, we combined optical coherence tomography (OCT) imaging system, fluorescence molecular imaging system and intravascular ultrasound (IVUS) system together. A tri-modality intravascular endoscope was built for multi-functional intravascular imaging study. This multi-modality endoscope was based on a combined OCT and fluorescence endoscope and a side view ultrasound transduce. A double clad fiber (DCF) combiner was used for OCT and fluorescence combined endoscope, single-mode core of the DCF was used to transmit both OCT and fluorescence excitation light, and the multimode inner cladding was used to collect fluorescence emission signal. A 35MHz side view ultrasound transducer was placed side by side with the optical probe. Ex vivo results showed that the integrated system could be used for early detection of vulnerable plaques.

8926-80, Session 16

Dual-modality optical frequency-domain (OFDI) and near-infrared fluorescence (NIRF) intravascular imaging: automated distance-based correction of NIRF signal intensity for quantitative molecular imaging

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Optical frequency-domain imaging (OFDI) is rapidly becoming the method of choice for the clinical intravascular assessment of coronary artery disease. Due to its very high axial resolution of ~10-15 ?m, OFDI visualizes the vessel wall microstructure in detail. Near-infrared fluorescence (NIRF) imaging is an emerging powerful method for molecular and functional imaging of biological tissues. The combination of NIRF molecular imaging with OFDI in a single catheter, would greatly expand the importance of intravascular imaging as a tool for both patient diagnosis and cardiovascular research.

One of the current challenges in the development of OFDI-NIRF multi-modality systems is that NIRF signal intensity attenuates as a function of the distance of the imaging catheter to the vessel wall. Generation of quantitative NIRF data requires accurate quantification of the vessel wall position in OFDI images. Given that multi-modality OFDI-NIRF systems acquire images at a very high frame-rate, typically a high number of images per pullback (>200) need to be analyzed. As such, manual analysis and calibration of NIRF data can be very time consuming, which is impractical for large clinical studies and unsuitable for real-time OFDI-NIRF image visualization.

In this study we describe a method for the automatic distance correction of NIRF data using OFDI morphological information. We developed an algorithm for the three-dimensional (3D) segmentation of vessel wall in OFDI images. Validation was performed by comparing automatic segmentation results to gold standard manual segmentation in a series of OFDI-NIRF datasets acquired in rabbits (n=5) in vivo. Good and suboptimal quality images were included in the analysis. A high Dice similarity coefficient was found between automatic and manual segmentation (>0.9). The processing-time (for a MATLAB® implementation on an 2011 iMac computer equipped with an i7 quad-core 3.4 GHz Intel processor, 12Gb of RAM and running OSX 10.6) was <0.1 second per image. These results suggest that this algorithm provides accurate and efficient NIRF signal intensity calibration.

8926-81, Session 16

OCT Paper

No Abstract Available

8926-82, Session 17

Comparison of spectroscopic photoacoustic imaging of human coronary atherosclerosis in two spectral bands

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Objectives: To investigate the performance of spectroscopic intravascular photoacoustic (sIVPA) imaging to detect and distinguish human coronary atherosclerotic lipids comparing excitation wavelengths near 1.2 µm and 1.7 µm.

Background: sIVPA generally utilizes one of the two high absorption bands in the lipid absorption spectrum at 1.2 µm and 1.7 µm that result from the second and first overtones of the C–H bond vibrations, respectively. The lipid absorption at 1.7 µm is 5.5 times higher than the absorption at 1.2 µm, but is accompanied by a roughly proportional increase in water absorption. Specific absorption signatures of various lipid compounds within the bands in either wavelength range can potentially be used to differentiate between plaque lipids and peri-adventitial lipids.

Methods: Combined sIVPA and intravascular ultrasound (IVUS) imaging at multiple wavelengths in both absorption bands was performed on a lipid containing vessel phantom and an atherosclerotic human coronary artery ex vivo. For each spectral range, we performed two tests: (1) Using co-registered data at two wavelengths, lipid maps were created to assess lipid sensitivity. (2) We correlated 7-wavelength IVPA phantom data with the absorption spectrum of cholesterol and peri-adventitial tissue, to investigate the ability to differentiate between plaque and peri-adventitial lipids, respectively.

Results: Lipid detection in a human atherosclerotic lesion with sIVPA required lower pulse energy at 1.7 µm than at 1.2 µm (0.4 mJ at the catheter tip, versus 1.2 mJ). The imaging depth was 5 mm at 1.7 µm versus 10 mm at 1.2 µm. In contrast, adequate differentiation between plaque and peri-adventitial lipids in a lipid containing vessel phantom could be achieved at 1.2 µm only.

Conclusions: The lipid-specific signal in a human coronary atherosclerotic plaque was stronger at 1.7 µm than at 1.2 µm, even though the pulse energy was lower. The penetration depth of the excitation light remains sufficient to image medium sized coronary arteries. Discrimination between plaque lipids and lipid deposits in peri-adventitial tissue was more successful at 1.2 µm.

8926-83, Session 17

Diagnostic accuracy of integrated optical coherence tomography and intravascular ultrasound (OCT-IVUS) system for coronary plaque characterization

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Intravascular ultrasound (IVUS) imaging and optical coherence tomography (OCT), two commonly used intracoronary imaging modalities, play important roles in plaque evaluation. The combined use of IVUS (visualize the entire plaque volume) and OCT (quantify the thickness of plaque cap, if any) is hypothesized to increase plaque diagnostic accuracy. Our group has developed a fully-integrated dual-modality OCT-IVUS imaging system and 3.6F catheter for simultaneous OCT-IVUS imaging, with a high resolution and deep penetration depth. However, the diagnostic accuracy of an integrated IVUS-OCT system has not been investigated. In this study, we imaged 102 coronary artery sites (over 200 regions of interest) from 15 cadavers, using our previous reported integrated IVUS-OCT system. IVUS-OCT images will be read by two skilled interventional cardiologists. Each region of interest will be classified as either a healthy region, calcification, lipid pool or fibrosis. Comparing the diagnosis by cardiologists using IVUS-OCT images with the diagnosis by the pathologist using gold-standard histological images, we will calculate the sensitivity and specificity for characterization of a healthy region, calcification, lipid pool or fibrosis of this integrated system. In vitro imaging of cadaver coronary specimens demonstrates the complementary nature of these two modalities for plaques classification. A higher accuracy is expected than using a single modality alone.

8926-84, Session 17

Intravascular ultrasound and photoacoustic imaging for atherosclerotic plaque characterization and local therapy guidance

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Combined intravascular ultrasound and photoacoustic (IVUS/IVPA) imaging has been investigated as a means of providing complimentary morphological and compositional assessment of atherosclerotic plaques, including localization of nanoscale contrast agents within macrophages. Our current research seeks to demonstrate the IVUS/IVPA imaging platform as a means of both delivering and monitoring locally targeted therapy within plaques through the use of multifunctional nanoparticles. An integrated IVUS/IVPA imaging catheter, coupling a single element ultrasound transducer and a side-looking optical fiber, was utilized to localize silica-coated gold nanorods (SiO2AuNR) within arterial tissue phantoms and ex vivo coronary artery sections. Linearity of temperature dependent changes in IVPA signal intensity was first verified by monitoring the IVPA signal obtained from SiO2AuNR inclusions in response to changes in temperature of the water tank. Next, a continuous wave (CW) laser source operating within the near infrared wavelength region was coupled into the optical fiber of the integrated IVUS/IVPA catheter in order to selectively heat SiO2AuNR, a process monitored by IVPA imaging. The incorporation of the CW laser source into the integrated catheter led to a measureable local temperature rise surrounding the SiO2AuNR, offering a potential method for delivering and tracking of targeted photothermal therapy. Additionally, photosensitizing agents were incorporated within the SiO2AuNR, creating multifunctional nanoparticles capable of inducing both photothermal and photodynamic therapeutic effects when irradiated using the IVUS/IVPA catheter. In conclusion, IVUS/IVPA imaging, in conjunction with nanoscale contrast agents, may be utilized as a platform for characterization and monitored treatment of atherosclerotic plaques during laser-induced phototherapy.

8926-85, Session 17

Bimodal imaging of ex-vivo human coronaries using a hybrid catheter combining fluorescence lifetime imaging (FLIm) and intravascular ultrasound (IVUS)

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This work reports on fluorescence lifetime imaging (FLIm) and intravascular ultrasound (IVUS) results from ex-vivo diseased human coronary arteries using a bimodal imaging catheter. The catheter includes a rotational 300 µm side-viewing fiber-optic (excitation source - Fianium, 355 nm, 10 KHz, 1.8 mJ/cm2) integrated with a conventional 3 Fr IVUS single element transducer (40 MHz, Boston Scientific). The catheter was used to perform sequential pullback imaging (1200 rpm, 2 mm/s) to assess biochemical and morphological features of the coronary vessel from FLIm and IVUS measurements respectively. Autofluorescence was measured at three emission wavelengths (CH1- 390/40, CH2- 452/45, CH3- 542?50 nm) corresponding to emission from collagen, elastin, and lipoproteins respectively. Co-registered FLIm, IVUS and histology data were obtained by imaging the vessel in a customized holder and using fiducial markers. Pathological features including fibroatheroma (FA), fibrocalcification (FC), and inflammatory cell infiltration (CD45+, CD68+) were identified in histology and the co-registered FLIm and IVUS data were studied. FLIm results showed lower lifetime values for lipid rich FA (CH1, 3.47±0.23 ns, CH2, 3.67±0.20 ns) compared to collagen rich fibrous tissue (CH1, 4.17±0.27 ns, CH2, 4.41±0.79 ns). Additionally, lifetime values also showed a difference between inflamed FA (CH1, 3.40±0.23 ns, CH2, 3.53±0.16 ns) and FA without inflammation (CH1, 4.13±0.30 ns, CH2, 4.01±0.56 ns). Integrated backscatter values from IVUS RF data were able to distinguish between hyperechoic fibrocalcified (-61.4±6.8 dB) and hypoechoic lipid pool (-81.1±4.5 dB) regions. Results demonstrate the ability of FLIm to complement IVUS imaging in characterizing plaque composition for intravascular diagnosis.

8926-86, Session 17

Bimodal imaging of atherosclerotic plaques: automated method for co-registration between fluorescence lifetime imaging and intravascular ultrasound data

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The risk of atherosclerotic plaque rupture cannot be assessed by the current imaging systems and thus new multi-modal technologies are under investigation. This includes combining a new fluorescence lifetime imaging (FLIm) technique, which is sensitive to plaque biochemical features, with conventional intravascular ultrasound (IVUS), which provides information on plaque morphology. In this study we present an automated method allowing for the co-registration of imaging data acquired based on these two techniques. Intraluminal studies were conducted in ex-vivo segments of human coronaries with a multimodal catheter integrating a commercial IVUS (40 MHz) and a rotational side-viewing fiber based multispectral FLIm system (355 nm excitation, 390±20, 452±22 and 542±25 nm acquisition wavelengths). The proposed method relies on the lumen/intima boundary extraction from the IVUS polar images. Image restoration is applied for the noise reduction and edge enhancement, while gray-scale peak tracing over the A-lines of the IVUS polar images is applied for the lumen boundary extraction. The detection of the guide-wire artifact is used for the angular registration between FLIm and IVUS data, after which the lifetime values can be mapped onto the segmented lumen/intima interface. The segmentation accuracy has been assessed against manual tracings, providing 0.107±0.007 mm mean Hausdorff distance. IVUS and FLIm co-registration was validated by observing changes in lifetime corresponding to changes in histology. This method makes the bi-modal FLIm and IVUS approach feasible for comprehensive intravascular diagnostic by providing co-registered biochemical and morphological information about atherosclerotic plaques.

8926-87, Session 17

Confocal acoustic radiation force optical coherence elastography for cardiovascular imaging

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Atherosclerosis is the main cause of cardiovascular disease and can lead to acute coronary events such as stroke and sudden death. The vulnerability and rupturing of the plaques are related to the stress on the fibrous cap, the cap thickness, and the composition of the plaques. Therefore it is important to measure the biomechanical properties of the artery tissue to monitor the atherosclerosis to reduce the rupture proneness of an artery.

In this study, we developed a confocal acoustic radiation force (ARF) associated optical coherence elastography (OCE) system using a phase-resolved method to dynamically evaluate the mechanical properties of artery tissue in order to identify plaques of different components. In this system, we used a ring ultrasound transducer to generate an oscillatory ARF as an internally localised vibrator to induce motions along the axial directions of the tissue and we detected the tissue motions by measuring the motion-induced phase changes between successive A lines, which were used to generate 2D phase maps to resolve the instantaneous tissue deformations. We performed experiments using this technique to measure the elastic properties both in phantoms and human atherosclerotic coronary arteries in vitro. The atherosclerosis was visible in the OCE image, but not obviously in the OCT image. The results verified that our ARF-OCE system has great potential to quantitatively measure the mechanical properties of artery tissue in vivo and to identify the composition of atherosclerotic lesions, which is of great importance for atherosclerosis diagnosis and treatment.

8926-88, Session 18

Validation of intracoronary OCT-Derived FFR for assessment of intermediate coronary lesions

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Objectives: The purpose of this study was to assess the functional significance of intermediate coronary artery lesions using the optical coherence tomography (OCT)-derived fractional flow reserve (FFR).

Methods: We identified 32 patients with intermediate coronary lesions in the OCT registry of Asan Medical Center, Korea, between 2011 and 2012, who underwent OCT and invasive physiologic assessment including FFR before treatment. FFR was measured at maximal hyperemia induced by intravenous adenosine. We randomly divided the patients into two groups: derivation (n=16) and validation (n=16) sets. Through the volumetric luminal analysis of OCT images, FFR was calculated from the blood flow resistance estimated using Poiseuille's law and the microvascular resistance optimized by the derivation set. We compared the calculated FFR to the pressure wire-measured FFR.

Results: The baseline clinical characteristics were similar in the derivation and validation sets. Angiographic diameter stenosis and the minimal luminal area (MLA) from OCT images were not different in the two groups. The calculated FFR values from the OCT images showed stronger linear correlation with the measured FFR values (derivation set: r=0.642; p=0.007; validation set: r=0.530; p=0.035) than OCT-derived MLA (r=0.409; p=0.020).

Conclusion: Our prediction method for the functional significance of intermediate coronary lesions derived from volumetric analysis of OCT images performed well in the small validation set.

8926-89, Session 18

Intracoronary polarization sensitive OCT

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Recently developed reconstruction algorithms enabled, for the first time, robust and reliable intracoronary polarization sensitive optical coherence tomography (PS-OCT). By overcoming system-induced polarization distortions, both measures of the sample birefringence and the sample depolarization properties became available, using conventional, inter-A-line modulated PS-OCT. We investigated these polarization signatures in atherosclerotic arteries of cadaveric human hearts, as well as in vivo in human patients and in a swine model of atherosclerosis. We previously reported on the sample birefringence in the intimal layer, which covers a wide range and corresponded well with collagen content assessed by histological analysis. The tunica media was found to feature a higher value of birefringence. Here, we used this good birefringence contrast, which exceeds the frequently poor intensity contrast between the intima and media, to facilitate automated segmentation of the intimal layer and advance on the quantitative analysis of collagen content within the intima using PS-OCT. Further, we found that an abrupt decrease in the degree of polarization was indicative of lipid pools. This provided a convenient contrast feature to segment the fibrous cap, and assess both its thickness and birefringence. Overall, intracoronary polarimetry measurements complement well the characterization of atherosclerotic coronary arteries with structural OCT, and might be able to help improve the patient management in the catheterization laboratory.

8926-90, Session 18

Intensity-based multidimensional flow measurements using intravascular optical frequency-domain imaging

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The assessment of the impairment of blood flow produced by a stenosis is critical to make decisions regarding the need to perform angioplasty or pharmacological treatment. Traditionally, this evaluation is carried out either using imaging techniques such as invasive coronary angiography, flow sensitive techniques such as Doppler ultrasound, or through surrogate indicators such as pressure measurements. In practice, intracoronary direct or indirect flow measurements have always been point-like with the use of Doppler or pressure wires. Multidimensional flow measurements could provide additional insight not only into the severity of a given lesion, but also into the pathogenesis of lesions and their relation to the presence of turbulent flow and non-laminar endothelial shear stress. We present the first steps toward intravascular flow measurements based on speckle decorrelation in catheter-based optical frequency-domain imaging (OFDI). Speckle decorrelation derives the flow-speed from the intensity fluctuations of speckle, and any intravascular OFDI system can be used to measure two- and three-dimensional flow profiles without the need of any hardware modification. We demonstrate two-dimensional flow profile reconstruction at 10 fps in a flow phantom setup, using intralipid at a concentration of 0.5% and parabolic laminar flow ranging from 0 mL / min to 80 mL / min in 3.2 mm-diameter tubing.

8926-91, Session 18

Analysis of intravascular OCT stent images using machine learning

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Intravascular OCT (iOCT) is the lone imaging modality with the resolution and contrast to provide in vivo assessments of tissue healing following stent implantation. Our Cardiovascular Imaging Core Laboratory has

served over 20 stent clinical trials, giving a large database of iOCT analyzed stents. Since each stent requires 6-16hrs of manual analysis, we are developing highly automated software to reduce effort and improve reproducibility. Using machine learning, physically meaningful image features, and leave-one-stent-out cross validation, software detected stent struts and classified each as covered, uncovered, or malapposed. Uncovered struts provide a potential biomarker for late stent thrombosis, a potentially life threatening event. To determine tissue coverage areas, we estimated stent "contours" by fitting detected struts to a periodic cubic spline. Tissue coverage area was obtained by subtracting lumen from stent area. For strut detection on 90 pullbacks, software gave recall=91±4% and precision=88±7%. Taking struts deemed not bright enough for manual analysis into consideration, precision improved to nearly 95%, approaching inter-observer variability. Differences in stent and tissue coverage areas were 0.27±0.54mm^2 and 0.23±0.55mm^2, respectively. Classification of uncovered versus covered struts was challenging, giving sensitivity=80%, specificity=82%, and ROC-AUC=0.88. Most errors were from struts with very thin tissue coverage (<30um). We are developing software to enable visualization, review, and editing of automated results, so as to provide comprehensive stent analysis for optimizing the myriad of stent design parameters, including drug, coatings, bioresorbable versus metal, etc.

8926-92, Session 19

Intravascular optical coherence tomography: automatic characterization of neointimal tissue maturity after stent implantation

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Intravascular optical coherence tomography (IVOCT) is rapidly becoming the method of choice for the assessment of vascular healing after stent implantation. With an axial resolution <20 ?m, IVOCT is capable of visualizing neointimal tissue covering stent struts and assessing "lack of coverage" which was shown to be a predictor of stent thrombosis. However, this is not the only parameter that needs to be assessed: the characterization of neointimal tissue maturity is also of importance especially in case of drug-eluting and bioresorbable stent devices. Immature tissue has been linked to delayed vascular healing and risk of stent thrombosis.

Previous studies indicated that well organized mature neointimal tissue appears as a high intensity, smooth and homogeneous region in IVOCT images, while lower intensity corresponds to immature tissue mainly composed by acellular material. However, neointimal tissue can currently be assessed only by a qualitative time-consuming manual analysis not suitable for on-line analysis and clinical practice.

In this study we present a method for the automatic characterization of neointimal tissue, based on texture analysis and supervised classification. The algorithm was trained and validated through the use of 53 IVOCT in-vivo images supported by histology data from an atherosclerotic animal model. A pixel-wise classification accuracy of 87% and a 2D region based accuracy of 92% (with sensitivity and specificity of 91% and 93%, respectively) were found. Combining this algorithm with previously developed routines for stent segmentation allows for a fully-automatic analysis of neointimal tissue. This may potentially expand the clinical value of IVOCT speeding-up current analysis methodologies potentially allowing for a wider use of this technology.

8926-93, Session 19

Optical coherence tomography tissue type (OC3T) imaging: clinical validation

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Detection of coronary vulnerable plaques is important for early diagnosis and better therapy of ischemic heart disease. We are developing optical coherence tomography (OCT) attenuation imaging as tissue characterization method. To investigate the ability of atherosclerotic tissue characterization in vivo, we are conducting the OC3T study: a prospective single-center validation study, comparing OCT-derived optical attenuation to near-infrared reflection spectroscopy and intravascular ultrasound (NIRS/IVUS; Infraredx TVC). Patient inclusion (N=80) was recently completed.

We analyze the OCT data to create color coded maps of optical attenuation. To facilitate the comparison with NIRS/IVUS, we transform the pullback attenuation data in a longitudinal/circumferential display (unrolling the cylinder that is the vessel wall) analogous to the NIRS Chemogram. This mapping display of OCT attenuation depicts lipid plaques in the entire pullback, sampling a user specified depth window from the lumen border.

A precise method for registration of the images from the two modalities for comparison consists of two steps, a primary matching procedure and a secondary elaborate non-linear registration. In the primary step, we align both maps, opened from the pericardial side, using the frame spacing/pitch of the modalities and the information of the biggest branch (such as LAD, LCX, RV branch, depending on the study vessel) to match its position in the two maps. In the secondary step we apply a polynomial transformation with control points on the branch vessels and calcification information to co-register the OCT map to the chemogram. This method may also be used for assessment of plaque progression/regression studies.

We will present a quantitative analysis of the registration procedure and discuss first results of the tissue type validation.

8926-94, Session 19

In vivo polarization-sensitive optical coherence tomography imaging in a murine model of myocardial infarction

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Objectives: The purpose of this study was to examine the birefringent characteristics of beating myocardium in a mouse myocardial infarction model using polarization-sensitive optical coherence tomography (PS-OCT).

Methods: Male C57B/6 mice (20-30g) were anesthetized with an intraperitoneal injection of zolazepam/tiletamine/xylazine mixture. After tracheal intubation, mice were mechanically ventilated and the left thoracotomy was performed. Left anterior descending artery was ligated with 7-0 silk suture. We imaged the anterior wall of left ventricle in a parasternal short axis window. The PS-OCT images were obtained in a beating heart before and after coronary artery ligation. The local birefringence images were compared between normal and infarcted myocardial tissues. For PS-OCT system, wavelength-swept laser with 140 nm bandwidth (center wavelength = 1,280 nm) and 120 kHz A-line

rate was used. Polarization modulator was used at the source output. The axial resolution was 6.8 micrometer and the sensitivity was 98.5dB.

Results: While the PS-OCT image showed weak and homogeneous birefringence in a normal myocardial tissue at both systole and diastole, we observed heterogeneous birefringence in an infarcted myocardial tissue. The thickness of homogeneous muscle layer was serially decreased after myocardial infarction.

Conclusion: We demonstrated in vivo PS-OCT images in a beating mouse heart and in a mouse model of myocardial infarction.

8926-95, Session 19

Foam cells and thin-cap fibroatheroma artifacts: optical coherence tomography versus histology

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Qualitative optical coherence tomography (OCT) plaque characterization methods are lacking. Here we present a quantitative study of macrophage optical properties and OCT vulnerable plaque features. Fresh human coronary arteries (n=8) were imaged in a mock catheterization laboratory. 60 regions of macrophages were identified: 44 caused bright spots, 8 caused bright spots with superficial shadowing (appeared as TCFA), and 8 caused shadows (appeared as lipid). Only 4 TCFAs were correctly identified. Our results show that current qualitative OCT plaque descriptions are confounded by artifacts and quantitative studies to recognize these three ways macrophages appear will improve vulnerable plaque imaging with OCT.

8926-96, Session 19

Optical properties of atherosclerotic tissue types from computational intravascular OCT

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We are developing computational approaches for tissue typing from coronary intravascular OCT (iOCT) 3D pullbacks. We measured optical properties (u,l) from small volumes of interest (VOIs with about 50 A-lines in r,?) identified by experts to be calcified, fibrotic, or lipid tissues. Model parameters of the imaging response to intralipid were estimated and used to correct clinical images from other catheters. Over 150 VOIs from 20 pullbacks were analyzed. Processing steps were: correct; take logarithm to enable usage of linear models and remove multiplicative noise; filter with average, median, or Lee filters having variable sized 3D windows; and estimate (u,l) from each VOI using fit to all data (ALL), average of fits to individual A-lines (AVG), parallel line fits (PL), robust average (RAVG), and robust parallel line (RPL). Following initial experiments, 15 combinations of filters/no filters/estimation were compared for chi-square, coefficient of variation of estimates, etc. Preliminary results included filtering is desirable, estimates varied with method, and RPL>RAVG>PL>AVG?ALL. RPL results were calcified (u=2.41±0.5/mm), fibrotic (u=1.46±0.11/mm), and lipid (u=4.38±0.15/mm), roughly similar to results in the literature. VOIs were drawn from intermediate r-depths, where any deviation from an “average” catheter correction was minimal. (u,l) feature space for these carefully selected VOIs showed separation of tissue types. Although 3D pullbacks present a challenge due to noise and view orientation, we believe that optical properties can be estimated and used in a comprehensive, computational approach to automated tissue classification.

8926-97, Session 20

Design and evaluation of optical fiber bundles for intracoronary laser speckle imaging

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Intracoronary Laser speckle imaging (ILSI) can provide information on the viscoelastic properties of coronary plaques by analyzing the dynamics of speckle intensity fluctuations. Recent studies conducted in living rabbits and swine have demonstrated the diagnostic capability of ILSI in detecting features of unstable plaques in vivo. To conduct ILSI, a small-diameter, flexible fiber bundle with low inter-fiber cross talk is needed to reliably transmit the speckle patterns reflected from the coronary wall in the presence of cardiac motion. In this study, we design and evaluate a new optical fiber bundle for ILSI to significantly minimize the modulation of speckle patterns caused by the inter-fiber crosstalk. We applied a theoretical model based on coupled mode theory and analyzed the influence of fiber core size, core spacing, numerical aperture (NA) and randomness in core size on mode coupling and speckle intensity modulation. We have further evaluated the influence of bending and twisting of fiber bundles on inter-fiber crosstalk and speckle pattern transmission. Our results show that in addition to large core-to-core separation, large refractive index contrast between core and cladding material and reduced number of propagating modes are essential for accurate speckle pattern transmission. We have designed and tested a new fiber bundle with 3?m core size, 8?m core spacing and 0.40 NA to conduct ILSI of coronary plaques at 690 nm. These studies provide solutions for optimizing fiber bundle selection for the reliable transmission of laser speckle patterns during the movement and twisting of fiber bundles for in vivo imaging.

8926-98, Session 20

Omni-directional viewing catheter for intravascular laser speckle imaging (ILSI)

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Acute myocardial infarction is frequently caused by the rupture of coronary plaques with severely compromised viscoelastic properties. We have developed a new optical technology termed intravascular laser speckle imaging (ILSI) that evaluates plaque viscoelastic properties, by measuring the Brownian motion of light scattering particles from temporally evolving laser speckle fluctuations. Our prior work demonstrated the feasibility of conducting ILSI in living animals via an intravascular catheter that permitted single point evaluation of the coronary wall. Here, we describe the development of an improved catheter for omni-directional illumination and viewing of the entire coronary circumference. The catheter incorporates custom-developed optics to illuminate and collect reflected speckle patterns from the coronary circumference. Time-varying speckle patterns are transmitted to a high-speed CMOS sensor via a GRIN lens and a low cross-talk fiber bundle incorporated within a 1mm diameter catheter. During imaging, the ILSI catheter can be pulled back through a custom-fabricated sheath to evaluate long coronary segments. To test the performance of the ILSI catheter, we collect speckle patterns from model arterial lesions created via lipid injection and thermal coagulation on a swine artery. The ILSI catheter can measure distinct differences in the time constant, ?,

of laser speckle fluctuations related with the viscoelastic properties of model lesions, with ?= 0.62 ± 0.19sec for fibrous lesions, 0.31 ± 0.15sec for the normal artery, and 0.18 ± 0.06sec for lipid lesions respectively. These results demonstrate the feasibility of conducting omni-directional evaluation of arterial mechanical properties using ILSI.

8926-99, Session PSun

Development of a fully integrated high-speed intravascular OFDI-NIRF imaging catheter system for detecting inflamed plaques in coronary sized vessels in vivo

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Optical frequency domain imaging (OFDI) is a second-generation form of optical coherence tomography (OCT). Intravascular OFDI allows to obtain 3D images of the microstructure of the artery wall in a few seconds. Near-infrared fluorescence (NIRF) enables molecular imaging of inflamed coronary plaques with fluorescence agent in vivo. We have developed a fully integrated, high-speed OFDI-NIRF imaging catheter system, which provides microstructural and molecular information from the artery wall simultaneously. Two imaging modalities were combined using a dichroic mirror and dual-band antireflection-coated optics. To ensure stable operation on high-speed rotation (100 rps), 1.8mm-diameter GRIN lens-based optical fiber coupling system and miniaturized rotary part were built. This high-speed rotary junction with a high-speed swept source (100 kHz) enables fast pullback up to 20 mm/sec. We acquired 3D microstructural images and 2D fluorescent images of the phantoms, which were built using agarose gel, intra-lipid, glass tubes, and fluorescent dye (Cy7) to verify the imaging performance of the developed imaging catheter. Also, we have acquired dual-modal images from inflamed atherosclerotic plaques using the fully integrated, high-speed OFDI-NIRF structural-molecular imaging with fluorescent agent. Atherosclerosis was induced in a rabbit model by balloon injury and a high-cholesterol diet for 12 weeks. In vivo dual-modal intravascular imaging was performed under saline flushing. NIRF signals and OFDI microstructures of atheromata were compared with corresponding histopathologic findings. The imaging catheter has the same physical properties with clinical imaging catheters. This highly translatable dual-modal imaging approach could enhance our capabilities to detect and study high-risk coronary plaques.

8926-100, Session PSun

Automatic detection of vessel lumen and stent struts in IV-OCT images to quantitatively estimate stent apposition and neointimal growth

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Intravascular optical coherence tomography (IV-OCT) is an emerging imaging technique to visualize microscopic structure of arterial wall and implanted devices in coronary vessels in vivo. Due to its high spatial resolution, it has become possible to precisely identify stent apposition and neointimal tissue growth after stent implantation. Quantitative analysis, which requires segmentation process, enables more accurate evaluation of intracoronary stents. However, it is still labor-intensive and time-consuming to manually segment vessel lumens and stent struts on hundreds of frames per each case. Thus, robust and fast automatic algorithm is urgently required. In this context, herein, we present an automatic segmentation algorithm for IV-OCT images. First, smoothing and simplification based on statistical filtering was performed for stent and lumen detection, respectively. Lumen was detected using A-line profiles. To increase robustness of the algorithm, lumen information of previous frame was also used. Then, guide-wire was detected and eliminated from further analysis. Shadow was detected based on intensity summation profile. Finally, stent struts were detected using derivative of each A-line. Whole procedure was developed as ImageJ plugin (NIH, Bethesda, MD, US). From clinical datasets, apposition and coverage were measured automatically and compared to manual segmentation. The sensitivity of this method was >90% for all measures (lumen, stent, and coverage) and the processing time was <0.5 sec per frame. The result shows that the presented method is robust and fast to automatically estimate stent apposition and coverage.

8926-101, Session PSun

Comparison of frequency domain optical coherence tomography and quantitative coronary angiography for the assessment of coronary lesions

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Frequency domain optical coherence tomography (FD-OCT) systems provide faster image acquisition speeds, higher frame rates, greater scan depths as compared to time domain OCT systems without the loss of resolution. Because of these features FD-OCT systems are now being widely used in modern catheterization laboratories worldwide. The main objective of this study is to highlight the potential of FD-OCT for the assessment of severe coronary artery stenosis. 38 coronary stenoses in 27 patients were assessed consecutively by FD-OCT, quantitative coronary angiography (QCA) and fractional flow reserve (FFR) during diagnostic coronary angiography. A commercially available frequency domain OCT system (C7XR) and the Dragonfly catheter (St. Jude Medical, Light lab Imaging Inc., Westford, Massachusetts) were used for the OCT imaging of the target stenosis. The minimal lumen area (MLA), minimal lumen diameter (MLD) and percent lumen area stenosis (%AS) were measured using FD-OCT. A comparison between FD-OCT and QCA derived measurements was performed using Bland-Altman analysis. The receiver operating characteristics (ROC) curve analysis was performed to assess the diagnostic efficiency of FD-OCT in identifying severe coronary stenosis as determined by FFR.

8926-102, Session PSun

Cardiac tissue characterization using near-infrared spectroscopy

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Treatments in radiofrequency ablation have often been limited by an inability to characterize tissues at sites of interest. In most cases, structural changes in tissue have been shown to express spectral

signatures that can be used to help describe underlying tissues. We demonstrate that changes in diffusely reflected near-infrared (NIR) light could serve as a method for differentiating types of cardiac tissue.

The setup employs a NIR LED (780-880 nm), a fiber optic probe, a spectrometer, and a computer. The fiber source-detector separation was measured to be 1.3 mm. A custom LabVIEW program facilitated system initialization and synchronized data acquisition.

Tissue from the left ventricular free wall was excised from a freshly extracted swine heart and submerged in temperature maintained phosphate buffered saline. A total of 1000 reflectance spectra were acquired from multiple locations for four different tissue types: 300 normal endocardium, 300 ablated endocardium, 200 normal epicardium, and 200 epicardial fat. Calibrated spectra from each group were fitted to a wavelength-dependent linear model and the slopes were extracted for comparison.

Bonferroni post-hoc analysis revealed significance in slope differences among normal and ablated endocardium ($p<.01$), normal epicardium and epicardial fat ($p<.01$), and normal endocardium and epicardium tissues ($p<.05$).

This study corroborates the feasibility of NIR spectroscopy for monitoring spectral changes at tissue states relevant to radiofrequency ablation therapy. In the future we will investigate methods such as the extraction of scattering and absorption coefficients, which may reveal more insight and also increase accuracy in spectral classification of tissues.

8926-103, Session 21

Efficient bone cutting with a novel diode pumped Er:YAG laser system: in vitro investigation and optimization of the treatment parameters *(Invited Paper)*

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It is well known that flashlamp pumped Er:YAG-lasers allow efficient bone ablation caused by the strong absorption at 3µm in water. Preliminary experiments showed that also the novel diode pumped Er:YAG laser system (Pantec Engineering AG) is an efficient tool for use in bone surgery.

The aim of the presented in vitro study is the investigation and optimization of the irradiation parameters and rinsing set-up to get high cutting quality and efficiency.

At first, optical simulations were performed to achieve a focus with homogeneous beam profile and variable diameter. An appropriate experimental set-up with beam delivery and focusing unit, a computer controlled stepper unit with sample holder, and a shutter unit was realized. So we are able to move the sample (1mm-10mm sawed slices of pig bone) with a defined velocity while irradiation by various laser parameters. Also we provided an appropriate water spray unit for rinsing the sample surface. The cuts were analyzed by light microcopy and laser scanning microscopy regarding the ablation quality and geometry, the ablation efficacy, and the thermal effects.

The results show that for efficient bone cutting not only a sharp focus (diameter about 200µm) with low NA and homogeneous beam profile is necessary. Also an appropriate rinsing unit with adapted parameters avoids both drying of the bone and too thick water layer.

In conclusion, these in vitro investigations demonstrate that high efficient bone cutting is possible with the diode pumped Er:YAG laser system using appropriate treatment set-up and parameters.

8926-104, Session 21

Laser technologies in treatment of degenerative-dystrophic bone diseases in children *(Invited Paper)*

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We present here 2 low invasive laser technologies for treatment of degenerative-dystrophic bone diseases in children. The first is transcutaneous laser osteoperforation developed by us and initially applied for treatment of acute purulent osteomyelitis1,2. The technology turned out to be rather effective in treatment of other bone diseases both inflammatory and traumatic: chronic osteomyelitides of different forms, osteal and osteoarticular panaritiums, delayed unions, false joints3,4. In 2009 we have presented preliminary results of laser osteoperforation application to aseptic osteonecrosis treatment4.

In this paper we present advanced results of this application. They are based on about 200 cases with aseptic necrosis of different bones: femoral head (Perthes disease) - 52% of the cases, 2nd metatarsal bone head (Kohler II disease) - 24%, patellar ligament at the tibial tuberosity (Osgood – Schlatter disease) - 20%, and calcaneal bone apophysis (Haglund-Schinz disease) - 4%.

The second technology is laser endocystic thermotherapy for treatment of bone cysts. The method was applied to 48 patients of 3-16 years old with aneurismal and solitary cysts of different localizations.

In both technologies a 970 nm diode laser was used. Results are good in majority of cases. Laser action has expressed ability to stimulate reparation processes in the bone tissue.

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8926-105, Session 21

Bone graft complications: what can we do to prevent them?

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Introduction:

Bone grafts are commonly used in oral & maxillofacial surgery, helping to restore missing bone structure and provide osseous support. In spite of their reported success, complications can and do arise. Examples include loosening and resorption of the graft, infection, and complete loss of the graft. These complications can potentially lead to larger defects, necessitating additional procedures to correct the problem. This not only causes great discomfort to the patient, but also drains considerable time and resources away from the clinician. Thus, improvements on identifying ways to identify and prevent these complications are constantly being sought. We have performed a literature review and identified several areas in the field of optics that could potentially help solve our problem.

Optical Techniques:

Raman spectroscopy has been shown to provide a transcutaneous measurement of bone mineral and matrix Raman bands. This could provide surgeons with the ability to more accurately assess bone graft osseointegration. In-vivo near-infrared optical imaging could potentially provide accurate diagnosis of pathologic lesions such as osteosarcoma. Contrast-enhanced ultrasound has been shown to detect vascular disturbances and other information related to the transplantation of osseous components.

Conclusion:

Bone graft complications can be one of the most devastating consequences of osseous surgery. As surgeons, we are constantly searching for ways to identify them earlier and prevent them. We hope that by presenting technologies currently being studied, we can gain a better insight to ways in which we can provide more accurate ways of assessing osseous grafts. By presenting these topics to the participating audience and garnering feedback, we hope to initiate dialogue and potential collaborative efforts.

8926-106, Session 21

RT-PCR standardization and bone mineralization after low-level laser therapy on adult osteoblast cells

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Purpose: Osteoblasts are capable to produce different compounds directly connected to bone mineralization process. This study aims to standardize the reverse transcriptase polymerase chain reaction (RT-PCR) for adult osteoblasts to observe the effect of low level laser therapy on bone mineralization. Methods: Five-millimeter fragments obtained from the mead femoral region of male Wistar rats were assigned into group A (n=10, laser) and group B (n=10, no laser), submitted to mechanic and enzymatic digestion. After 7 days, cultures of group A were irradiated daily on a single spot with a GaInAs laser, ?=808nm, 200mW/cm2, 2J/cm2, bean diameter of 0,02mm, 5 seconds for 6 days. Group B was manipulated but received no laser irradiation. After 13 days the cells were trypsinized for 15 minute and stabilized with RNA later® for RNA extraction with Trizol®. cDNA synthesis used 10µg of RNA and M-MLV® enzyme. PCR was accomplished using the ?-actin gene as a control. Another aliquot was fixed for Hematoxylin-Eosin and Von Kossa staining to visualize bone mineralization areas. Results: Under UV light we observed clearly the amplification of ?-actin gene around 400bp. HE and Von Kossa staining showed osteoblast clusters, a higher number of bone cells and well defined mineralization areas in group A. Conclusion: The cell culture, RNA extraction and RT-PCR method for adult osteoblasts was effective, allowing to use these methods for bone mineralization studies. Laser improved bone mineralization and further studies are needed involving osteogenesis, calcium release mechanisms and calcium related channels.

8926-107, Session 21

Radiofrequency ablation for oral and maxillofacial pathologies: A description of the technique *(Invited Paper)*

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Introduction: Radiofrequency ablation (RFA) refers to a high-frequency current that heats and coagulates tissue. In the standard RFA setup, three components are used: a generator, an active electrode, and a dispersive electrode. RFA has garnered support in many of the surgical fields as an alternative to traditional procedures used in tumor removal. Other methods can prove to be more invasive and disfiguring to the patient, in addition to the unwarranted side effects; however, RFA provides a more localized treatment, resulting in decreased co-morbidity to the patient. Currently, its use in the field of oral & maxillofacial surgery is limited, as its technology has not reached our field. By describing its limited use to the optics community, we hope to expand its uses and provide patients with one more alternative treatment option.

Methods and Uses: We will describe the use of RFA on three types of pathology: lymphangioma, rhabdomyoscarcoma, and oral squamous cell carcinoma. The majority of treatments geared towards these pathologies involve surgical resection, followed by reconstruction. However, damage to vital structures coupled with esthetic disfigurement makes RFA a more valuable alternative. In many of the cases, the tumors were successfully removed without recurrence.

Conclusion: While the use of RFA has been scarce in our field, we believe that with more exposure it can gain momentum as an alternative to current treatment options. However, there are improvements that we feel can be made, helping to maximize its effectiveness.

8926-108, Session 22

Photothermal coherence tomographies for hard tissue imaging *(Invited Paper)*

Andreas Mandelis, Nima Tabatabaei, Univ. of Toronto (Canada)

In this talk two novel thermal-wave imaging methodologies will be presented: Matched filter binary phase coded Photothermal Coherence Tomography (BPC-PCT) and truncated-correlation photothermal coherence tomography (TC-PCT). The physical principles of these methodologies and examples of imaging applications to bone imaging will be discussed.

First, thermophotonic radar imaging principles and techniques using chirped or BPC modulation will be introduced. These are methods which can break through the maximum detection depth/depth resolution limitations of conventional photothermal waves. Using matched-filter principles, BPC-PCT, a methodology enabling parabolic diffusion-wave energy fields to exhibit energy localization akin to propagating hyperbolic wave-fields will be described [1]. It allows for deconvolution of individual responses of superposed axially discrete sources, opening a new field: depth-resolved thermal coherence tomography. Several examples from dental enamel caries diagnostic imaging to cortical and trabecular bone imaging will be discussed.

Next, truncated-correlation photothermal coherence tomography (TC-PCT) [2] will be presented, which exhibits the highest degree of energy localization and image resolution in a parabolic diffusion wave field to-date. TC-PCT enables three-dimensional “crisp” visualization of subsurface features/discontinuities which is not otherwise possible with known optical or conventional photothermal imaging techniques. Examples to be presented include trabecular bone structure through cortical and soft tissue overlayers, structural changes in animal bones following demineralization induced bone loss (artificial osteoporosis), and burn depth profiles in tissues. As a consequence of its high axial resolution and nearly lossless character, TC-PCT exhibits sub-surface depth profilometric capabilities over several (~ 4) thermal diffusion lengths, well beyond those of today’s thermal-wave probes.

8926-109, Session 22

Deep tissue imaging of micro-fracture and non-displaced fracture of bone using the three near-infrared therapeutic windows *(Invited Paper)*

Laura A. Sordillo, Yang Pu, Peter P. Sordillo, Yuri Budansky, Robert R. Alfano, The City College of New York (United States)

Near-infrared (NIR) light has three (3) NIR therapeutic windows, which allow for deeper depth penetration in tissue. It is well-known that optical images of tissue are distorted due to light scattering from cells and intracellular matrix, and from absorption of water chromophores within tissue. Using the first, second, and third NIR therapeutic window, we investigated results from images of chicken bone with micro-fractures and non-displaced fractures with and without tissue. Images show scattering light in the third NIR window with wavelengths between 1,650 nm and 1,840 nm is diminished and absorption is increased slightly from conventional first and second NIR windows.

8926-110, Session 22

Human bone periosteum studied by one: photon and two-photon fluorescence

Noella Hatak, St. John's Univ. (United States); Nanda Shivananjappa, Yang Pu, Lingyan Shi, Stephanie Lubicz M.D., Robert R. Alfano, The City College of New York (United States)

Bone diseases leading to bone loss are more prevalent in both developed and underdeveloped countries. The minerals of the bone are held together in a tightly woven complex of collagen and osteocytes. Minerals of the bone can be lost due to demineralization or due to discrepancies in the complexes. Optical spectroscopy experiments are conducted on the periosteum, the outermost layer of the bone, along with collagen and NADH powders . The fluorescence spectra and two photon (2P) fluorescence images at various depths are used to show the different structures of the periosteum layer up to 70 um with emission at 465 nanometers from mostly collagen using a Parrie 2P femtosecond laser microscope. A Perkin Elmer spectrometer LS50 was used for the 1P spectra to confirm collagen . The experiments revealed that bone has maximum 1P intensity at 485 nanometers associated from collagen and bone. Fluorescence studies on collagen in vitro suggest that the ranges obtained in 2P image can be used for observed structure diagnosis and treatment of diseases.

The periosteum is the layer of tissue on the external surface of most bones. It is made up of three distinct layers ; however, this study is focused on the outermost layer which is primarily made up of collagen followed by fibroblast and osteoblast . The periosteum is easy to evaluate because of its location on the surface of bones and presents an opportunity to study the intermediary products before the process of mineralization occurs. Collagen is the basic component for the makeup of bone. Its main property is to provide flexibility and structure. A study of the makeup of the periosteum in the 2P images allows for a greater understanding of the chain of activities leading to the production of bone and changes in its structures of the fibroblast and Osteoblast from 0 to 70 um . With new 2P and 1P spectral data bone , diseases and porosity can be better understood which can lead to new discoveries in preventing them. This preliminary report shows how 1P and 2P images can yield information on structure at optical properties of bone and more specifically the periosteum.

8926-111, Session 23

What can optical techniques tell us about the 3D collagen structure of articular cartilage? *(Invited Paper)*

Stephen J. Matcher, The Univ. of Sheffield (United Kingdom)

Although formally not a calcified bony tissue, articular cartilage is nonetheless an extremely important functional extension of bone, which serves to minimize friction at the sliding interface between two bone epiphyses within an articular joint. Alterations in the biology and biotribology of cartilage are heavily implicated in chronic conditions such as osteoarthritis. The 3-D architecture of collagen in articular cartilage is believed to be a strong modulator of the mechanical properties of cartilage.

The anatomical structure of articular cartilage has been the subject of study for over a century. Early studies exploited the optical birefringence of collagen to study thin sections however such 2-D sections through a 3-D structure coupled with a lack of quantification led to only a partial picture. Cartilage structure was defined in terms of three zones, the "superficial", "transitional" and "radial" zones. Cryo-SEM imaging provides new insights but again is fundamentally 2-D in nature. Modern approaches such as diffusion-tensor MRI can potentially address some of these limitations but DT-MRI can struggle in low anisotropy tissues such as cartilage. In recent years groups have applied quantitative polarized light microscopy to study the thickness of the three classic cartilage zones.

In this talk I will review the results obtained by optical techniques and what they tell us about cartilage structure. I will highlight some recent work from my own lab where we use multi-angle OCT to perform non-destructive 3-D polarimetry on cartilage. Some conclusions about possible collagen architectures in cartilage are drawn.

8926-112, Session 23

Infrared fiber optic probes for evaluation of musculoskeletal tissue pathology *(Invited Paper)*

Mugdha Padalkar, Cushla McGoverin, Quam Onigbanjo, Temple University (United States); Richard G Spencer, National Institutes of Health (United States); Scott Barbash, Eric Kropf, Temple University School of Medicine (United States); Nancy Pleshko, Temple University (United States)

Osteoarthritis (OA) is a debilitating and prevalent disease characterized by cartilage degeneration. Early detection remains elusive, with current imaging methods lacking adequate sensitivity to pathologic cartilage changes. Our approach is through use of the complementary techniques of mid- and near- infrared (IR) spectroscopy using arthroscopic-based fiber-optic devices. Mid-IR spectra (800 – 4000 cm-1) exhibit quantifiable molecular absorbances, although penetration depth is only ~ 10 microns. Near-IR (4000 – 8000 cm-1) penetrates to ~cm, but with the exception of the water band, exhibits complex overlapping bands that are not readily quantifiable. However, we have found that combined mid- and near-IR analysis greatly extends the information available through either in analysis of cartilage, ligaments and tendons. We discuss our basic science studies and translation to clinical research with novel arthroscopic probes.

8926-113, Session 23

Photoacoustic and ultrasound backscattering characterization of bone tissue

Bahman Lashkari, Lifeng Yang, Joel W. Y. Tan, Andreas Mandelis, Univ. of Toronto (Canada)

Osteoporosis is a major public health problem in many countries. Fortunately new treatments have been developed and diagnostic methods are also improving. One important issue is to offer an inexpensive and reliable method that can assess many/all aspects of bone health. Our preliminary studies demonstrated the feasibility of photoacoustic (PA) detection of bone density variations. In the reported studies, we used back-scattered ultrasound (US) and back-propagating PA to characterize in-vitro animal bone samples. US is sensitive to mechanical properties, and PA is sensitive to optical and acoustic properties; each modality reveals different aspects of bone health and integrity. Both modalities were applied in the frequency-domain (FD), employing linear frequency modulation chirps. The use of FD signals facilitates the spectral analysis and comparison between the modalities.

To simulate the variation in the bone due the disease, samples were either decalcified by mild ethylenediaminetetraacetic acid (EDTA) solution or decollagenized by sodium hypochlorite solutions. The sensitivity of both modalities was examined by comparing the parameters before and after demineralizing and decollagenizing the bone samples. The samples were not only extracted from cancellous bone parts but we examined the sensitivity of both modalities to minor changes in the cortical layer as well. Back-propagation measurements on cortical layer facilitate the assessment of bone health in different skeletal sites and overcome the challenge of laser penetration in deep hard tissue. The higher sensitivity of the co-registered PA and US modalities may provide a viable in-vivo technique for monitoring osteoporosis.

8926-114, Session 23

Photonic hydrogel beads for controlled release of risedronate

Deepak Khajuria, D. Roy Mahapatra, Indian Institute of Science (India)

Hydrogel nanoparticles are having increasing applications in biological sensing, drug delivery, and tissue regeneration. Hydrogels can protect the drug from hostile environments, e.g., the presence of enzymes and low pH in the stomach. Among several types of hydrogels, temperature and pH-sensitive hydrogels are the most widely investigated ones. Osteoporosis and Paget's disease of bone are major problems in women and geriatric patients. Antiresorptive agents are normally recommended in such case. Despite their benefits, bisphosphonate based drug molecules suffer from very poor oral bioavailability (1–2%). Risedronate, which is used clinically to treat osteoporosis, shows less than 1% bioavailability in humans due to the low absorption in gastrointestinal tract. Extensive ionization and highly hydrophilic nature of bisphosphonates prevent it from transcellular transport across intestinal epithelium and favor the paracellular route. Higher localized concentration of bisphosphonates has resulted in severe gastro-intestinal side effects such as dysphagia, esophagitis and gastric ulceration. It is of great importance therefore to understand the mechanism of release and transport of Bisphosphonates. Photonic tagging while performing in-vitro tests on microfluidic assays simulating the gastro-intestinal cellular conditions is of main focus in this work. We prepared photonically tagged risedronate-alginate complex bead from solution of risedronate and alginate with photo-reactive molecules. Uptake and release of risedronate are then studied. After loading risedronates into hydrogel, several parameters that have certain influences on drug loading and release efficiency were modified and traced using the photonic tags in a microfluidic assay under microscope.

8926-115, Session 24

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic analysis of regenerated bone

Carolina Benetti, Instituto de Pesquisas Energéticas e Nucleares (Brazil); Sergei G. Kazarain, Imperial College London (United Kingdom); Marco A. V. Alves, Alberto Blay, Luciana Correa, Univ. de São Paulo (Brazil); Denise M. Zezell, Instituto de Pesquisas Energéticas e Nucleares (Brazil)

The cutting of bone is routinely required in medical procedures, for instance, in dental applications. In such cases, bone regeneration and new bone quality can determine the success of treatment. This study investigated the main spectral differences of undamaged and healed bone using the ATR-FTIR spectroscopy imaging technique. Three rabbits were submitted to a surgical procedure; a small piece of bone (3?3 mm2) was removed from both sides of their jaws using a high speed drill. After 15 days, the rabbits were euthanized and the jaws were removed. A sample was cut from each side of the jaw containing regions of undamaged and newly formed bone, resulting in six samples which were polished for spectroscopic comparison. The samples were analyzed in an FTIR spectrometer (Varian 670 FTIR) via a diamond ATR imaging Golden Gate™ (Specac) and an FPA detector. Spectral characteristics were compared and particular attention was paid to the proportion of phosphate to amide I bands and the width of the phosphate band. The results show that the ratio of phosphate to amide I is smaller in new bone tissue than in the undamaged bone, indicating a higher organic content in the newly formed bone. The analysis of the width of the phosphate band suggests a crystallinity difference between both tissues, since the width was higher in the new bone than in the natural bone. These results suggest that the differences observed in bone aging processes by FTIR spectroscopic can be applied to the study of healing processes.

8926-116, Session 24

Spatial frequencies from human bone periosteum at different depths using two-photon microscopic images

Laura A. Sordillo, Stephen Bhagroo, Theinan Nguyen, Lingyan Shi, Noella Hatak, Stephanie Lubicz M.D., Yang Pu, Robert R. Alfano, The City College of New York (United States)

The periosteum is a fibrous membrane covering the surface of bone. The spatial frequencies from images acquired with two-photon (2P) excitation microscopy of the layers of the periosteum of human bone at different depths were investigated. High contrast images of collagen present in the periosteum at different depths were obtained. The spatial frequency spectra from the outer and inner periosteal region show significant spectral peak differences which can provide information on the structure of the layers of the periosteum.

8926-117, Session 24

Photoacoustic and ultrasound dual-modality imaging for inflammatory arthritis *(Invited Paper)*

Guan Xu, Univ. of Michigan Medical School (United States); David L. Chamberland, Univ. of Michigan (United States); Gandikota Girish, Xueding Wang, Univ. of Michigan Medical School (United States)

Arthritis is a leading cause of disability, affecting 46 million of the population in the U.S. Rendering new optical contrast in articular tissues at high spatial and temporal resolution, emerging photoacoustic imaging (PAI) combined with more established ultrasound (US) technologies provides unique opportunities for diagnosis and treatment monitoring of inflammatory arthritis. In addition to capturing peripheral bone and soft tissue images, PAI has the capability to quantify hemodynamic properties including regional blood oxygenation and blood volume, both abnormal in synovial tissues affected by arthritis. Therefore, PAI, together with US, should be of considerable help for further understanding the pathophysiology of arthritis as well as assisting in therapeutic decisions, including assessing the efficacy of new pharmacological therapies. This talk will review our work during the last six years at the Department of Radiology in the University of Michigan. It will include the first imaging findings on animal models of arthritis, including both chronic and acute inflammation models. Validation of its performance in monitoring and objective evaluation of pharmacological therapy on animal model of arthritis will also be presented. Moreover, our resent work on PAI and US imaging of human peripheral joints realized with a commercial US platform will also be presented. This very initial clinical trial is based on our latest version of a truly real-time dual-modality imaging system, performed in the same way as in current clinic. A few patient scanning cases on metacarpophalangeal and proximal interphalangeal joints validated our hypothesis that PAI can identify functional biomarkers associated with arthritis.

8926-139, Session 24

Collagen density determines impaired mechanical function of osteogenesis imperfecta: a murine model study

Hao Ding, Catherine Ambrose, The Univ. of Texas Health Science Ctr. at Houston (United States); Ingo Grafe, Brendan Lee, Baylor Univ. (United States); Xiaohong Bi, The Univ. of Texas Health Science Ctr. at Houston (United States)

Osteogenesis Imperfecta (OI) is a genetic disorder of bone organic matrix, characterized by a brittle and deformed skeleton. Bone composition, an important determinant of fracture resistance, has been investigated in various animal models of OI using FTIR imaging and Raman spectroscopy. Mineralization, mineral crystallinity and carbonation are the most frequently reported parameters in the previous studies. The results however can be contradictory depending on the age of the animals. Furthermore, no correlations have been performed between the compositional properties and the mechanical function in these models. In the current study, we investigated the material properties of a murine model with OI phenotype, and identified a spectral marker of organic matrix that is significantly correlated to bone mechanical properties in this model.

The femurs from 16 weeks old female CRTAP knockout mice (n = 6) and wild-type control (n = 7) were harvested and soft tissues removed. Three point bending tests were performed for material property evaluation. Raman spectra were collected from the cortical surface of the same femurs. Decreased mineral crystallinity and carbonate/matrix ratio were observed with OI ($p<0.05$). Mineral/matrix and carbonate/phosphate ratios did not show a significant difference between the OI mice and the wildtype controls. Collagen/matrix, or collagen density, calculated by the peak height ratios of proline and amide 1, decreased with OI ($p<0.001$), and significantly correlated to bone mechanical properties ($p<0.001$). This study indicates that the impaired mechanical function in OI mice results from decreased collagen density, but not mineral properties.

8927-1, Session 1

Pathology of pediatric GI disorders *(Invited Paper)*

Guillermo J. Tearney M.D., Wellman Ctr. for Photomedicine (United States)

No Abstract Available

8927-2, Session 1

In vivo large-area confocal imaging of esophagus using SECM

DongKyun Kang, Massachusetts General Hospital (United States); Simon Schlachter, Massachusetts General Hospital (United States) and Nine Point Medical (United States); Robert W. Carruth, Massachusetts General Hospital (United States); Minkyu Kim, Massachusetts General Hospital (United States) and Tokyo Univ. (Japan); Tao Wu, Wellman Ctr. for Photomedicine (United States); Nima Tabatabaei, Massachusetts General Hospital (United States); Paulino Vacas-Jacques, Milen Shishkov, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Kevin Woods, Emory Univ. Hospital (United States); Jenny S. Sauk, John Leung, Norman S. Nishioka M.D., Massachusetts General Hospital (United States); Guillermo J. Tearney M.D., Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States)

Spectrally encoded confocal microscopy (SECM) is a form of reflectance confocal microscopy that can achieve very high imaging speed in relatively simple probe optics. For endoscopic imaging of a luminal organ such as the esophagus, SECM can be configured as a side-looking probe, and the SECM probe can be helically scanned to image a large region of the tissue. Here, we present in vivo large-area confocal imaging of the esophagus using SECM. We developed an endoscopic probe that is specially tailored for the SECM esophageal imaging. The probe used a water-immersion aspheric lens (NA = 0.5) as the objective lens to achieve good tissue imaging performance. The objective lens was tilted relative to the tissue surface to conduct volumetric imaging during a single helical scan of the probe. The probe had the diameter of 5.85mm and the rigid length of 30 mm. The resolution was 2.3 μm and 17 μm for the lateral and axial directions, respectively. The probe was tested in a living swine. 6% acetic acid was sprayed on the esophagus to enhance the nuclear contrast prior to the SECM imaging. A 5-cm-long segment of the swine esophagus was imaged in about 2 minutes. The low-magnification view of the swine esophagus image visualized numerous papillae and the border between the epithelium and lamina propria. The high-magnification view clearly visualized cellular details of the esophagus: nuclei in the superficial epithelium, basal cell nuclei, and papillae. Volumetric imaging capability was demonstrated by showing the cellular morphology changes at different depth levels. The results showed that SECM can successfully conduct large-area imaging of the esophagus in vivo.

8927-3, Session 1

Flow velocity measurements in spectrally encoded flow cytometry

Tal Elhanan, Lior Golan, Dvir Yelin, Technion-Israel Institute of Technology (Israel)

Using a diffraction grating and a high numerical-aperture objective lens, spectrally encoded flow cytometry (SEFC) allows two-dimensional in vivo microscopy of flowing blood cells noninvasively and without exogenous labeling. By imaging blood cells in small capillary vessels in the oral mucosa, SEFC provides useful clinical information on blood cell count, cell morphology and size distribution. Yet, effective quantification of the flow images requires knowledge of the exact speed of the cells, and while flow velocity is not necessarily an important parameter on its own, it permits accurate scaling of the time axis, improving accuracy and consistency of the blood counting parameters. In this work, we demonstrate two techniques for measuring flow velocity. The first measures the apparent ellipticity of white blood cells and uses the deviation from a perfect circle for scaling the time (flow) axis. The second technique uses a simple optical add-on apparatus that splits the broadband light into two separate optical paths. By analyzing the scrambled image obtained from the two spectral lines simultaneously we compute the flow velocity at each point across the image. We demonstrate measurements of important blood parameters with greater accuracy compared to previous software-based methods. The velocity data from the two techniques was in good agreement with the known velocity calculated from the known pump specifications and the cross section of the flow chamber. In vivo velocity estimation has also been demonstrated, allowing accurate and continuous blood counting.

8927-4, Session 1

Development of tissue marking system for image-guided biopsy in spectrally-encoded confocal endomicroscopy platform

Nima Tabatabaei, Harvard Medical School (United States) and Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Minkyu Kim, Harvard Medical School (United States) and Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and The Univ. of Tokyo (Japan); Tao Wu, Dongkyun Kang, Guillermo J. Tearney M.D., Harvard Medical School (United States) and Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States)

In current standard of care, the diagnosis and surveillance of several esophageal diseases are accomplished by random endoscopic biopsy. Since random biopsies only represent a limited fraction of the esophagus, this procedure is prone to sampling error and results in low diagnostic yield. Spectrally-encoded confocal microscopy (SECM) is a reflectance microscopy technique which has been implemented in probe and tethered-capsule arrangements for in-vivo sub-cellular imaging of the esophagus. Here we describe the integration of laser marking into the SECM system for image-guided biopsy for the first time. This platform allows the operator to select the target region of interest (ROI) from the SECM dataset in real time and place two marks on either sides of the ROI to aid the subsequent endoscopic biopsy. In the marking system, the optical power from 4 pump laser diodes (2@1430nm and 2@1450nm) are added by polarization combining and wavelength-division multiplexing in a single-mode fiber and eventually integrated to the SECM imaging system with wavelength-division multiplexing. At these wavelengths water absorption is relatively high, while the optical path through the SECM optics is identical to that of the imaging laser. To determine the optimal laser exposure parameters, four-quadrant SECM marking and imaging will be carried out on the esophageal mucosa of Yorkshire swine in increasing exposure durations at 2-cm intervals. Then, visibility of the marks will be studied by video endoscopy and maximum injury depth will be quantified by Nitro-blue tetrazolium chloride histology of corresponding biopsies. Finally, the system will be evaluated by SECM marking and imaging in humans.

8927-5, Session 1

Spectrally-encoded confocal endomicroscopy capsule for diagnosis of eosinophilic esophagitis

Nima Tabatabaei, Dongkyun Kang, Tao Wu, Harvard Medical School (United States) and Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Minkyu Kim, Harvard Medical School (United States) and Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and The Univ. of Tokyo (Japan); Robert W. Carruth, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Guillermo J. Tearney M.D., Harvard Medical School (United States) and Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States)

Eosinophilic esophagitis (EoE) is a prevalent food allergy disorder that manifests by eosinophilic infiltration within the esophageal wall. Current standard of care for treatment of the disease requires several follow up sedated endoscopies with biopsies to confirm elimination of eosinophils. These procedures are expensive, time consuming, and difficult for patients to tolerate. Spectrally-encoded confocal microscopy (SECM) is a reflectance microscopy technique which assigns the wavelength components of a wavelength swept laser light spatially across a focal line inside the sample. In a prior bench top study, we have shown that SECM is capable of identifying eosinophils in biopsy samples. Here we describe the implementation of SECM in a miniaturized, tethered capsule which is capable of performing sub-cellular imaging of the esophagus in unsedated patients. The capsule is filled with water and made out of fluorinated ethylene propylene (refractive index = 1.338) to maintain continuity of index of refraction. Single mode lensed fiber with a NA of 0.18 at 1300nm is used to shorten the length of the 7 mm diameter capsule to 30 mm. Experiments carried out indicate transverse and axial resolutions of 2 µm and 12 µm, respectively. The spectrally encoded field of view is approximately 300µm, which is close to the dimensions of the high power field used for histopathologic diagnosis of EoE. Preclinical in-vivo animal studies as well as ex-vivo imaging of de-identified fresh biopsy samples from EoE patients confirmed the capability of the capsule to visualize eosinophils. The results of the first human study on normal and EoE patients will be presented.

8927-7, Session 2

Development and characterisation of wide-field and confocal fluorescence lifetime imaging endoscopes for biomedical applications

Hugh Sparks, Ian H. Munro, Gordon Thomas Kennedy, Imperial College London (United Kingdom); Eishu Hirata, Cancer Research UK London Research Institute (United Kingdom); Ezra Nigar, The North West London Hospitals NHS Trust (United Kingdom); Sean Warren, Imperial College London (United Kingdom); Erik Sahai, Cancer Research UK London Research Institute (United Kingdom); Taran S. Tatla, Northwick Park Hospital (United Kingdom); Chris Dunsby, Paul M. W. French, Imperial College London (United Kingdom)

FLIM can provide useful molecular contrast when imaging tissue autofluorescence for label-free readouts of disease and for preclinical imaging of labelled tissues. For minimally invasive access to internal organs in vivo, it is desirable to develop FLIM endoscopes. We present a wide-field flexible FLIM endoscope using compact pulsed excitation sources with time-gated imaging for both FLIM of FRET in disease models and clinical imaging of tissue autofluorescence. This utilises a flexible coherent fiber bundle (2.3 mm diameter) that presents a spatially varying instrument response function (IRF) caused by modal dispersion in the excitation optical fiber that we have corrected in software. We will present preliminary ex vivo wide-field FLIM of human head and neck lesions and progress towards endoscopy.

We also present a scanning confocal FLIM endomicroscope utilising time-correlated single photon counting (TCSPC) that we are developing for in vivo quantitative imaging of fluorescence resonance energy transfer (FRET). This is based in the Mauna Kea Technologies Cellvizio system that provides internal access via an endoscope of less than 2.6 mm diameter. TCSPC electronics run in a first-in first-out (FIFO) mode to enable live streaming of lifetime maps. We show that it is necessary to account for both variations in count rate dependent background across the fibre bundle (caused by background fluorescence from the fibre bundle) and material dispersion effects in order to achieve accurate fluorescence decay analysis. We will present our work towards in vivo imaging of murine xenograft models of ovarian cancer using CFP- and GFP-based FRET sensors.

8927-8, Session 2

Comparing line-scanned and point-scanned dual-axis confocal microscope performance in phantoms and tissues for high-speed point-of-care pathology

Danni Wang, Ye Chen, Jonathan T. Liu, Stony Brook Univ. (United States)

The point-scanning dual-axis confocal (PS-DAC) microscope has been shown to exhibit a superior capability to reject out-of-focus and multiply scattered light in comparison to its conventional single-axis counterpart. However, the slow frame-rate resulting from point-by-point data collection makes these systems vulnerable to motion artifacts. A line-scanning dual-axis confocal (LS-DAC) microscope is potentially capable of high speed (video-rate) imaging through line-by-line data collection. Here we evaluate the performance trade-offs between a LS-DAC to PS-DAC microscope with identical spatial resolutions. Characterization experiments of the LS-DAC and PS-DAC microscopes with tissue phantoms, in reflectance mode, are shown to match results from Monte-Carlo scattering simulations of the systems. Fluorescent images of mouse brain vasculature, obtained using resolution-matched LS-DAC and PS-DAC microscopes, demonstrate the comparable performance of LS-DAC and PS-DAC microscopy at shallow depths.

8927-9, Session 2

Development and testing of an achromatized miniature objective for in vivo confocal microendoscopy

Tzu-Yu Wu, Arthur F. Gmitro, College of Optical Sciences, The Univ. of Arizona (United States); Andrew R. Rouse, The Univ. of Arizona (United States)

Confocal microendoscopy provides in vivo microscopic visualization of cellular structures, enabling the diagnosis of epithelial surfaces of internal organs in real time. An advanced miniature objective is typically required for confocal microendoscopy. We previously presented a design of a miniature water-immersion microscope objective that delivers nearly diffraction-limited performance over a broad spectral range of 486 nm -1 µm. The miniature objective is designed for, but not limited to, the use in a flexible fiber-bundle-based fluorescence confocal microendoscope that can be routed through the therapeutic channel of a standard wide view endoscope, adding microscopic imaging capability to a conventional endoscope. Continued development has been followed by a significant

optical engineering stage to bring the 0.6 NA, 488 µm field of view, 2.1 magnification lens design into a clinically viable mechanical assembly. Here we present the stray light analysis, mechanical designs for mounting and housing the lens elements, and optical-bench test results of the fabricated miniature objective. A modulation transfer function (MTF) value of 65% at the Nyquist frequency of a 50,000- element fiber-optic imaging bundle has been obtained from a slanted edge MTF test. Early ex vivo results of human esophageal tissues imaged by the miniature objective as a stand-alone optical system and integrated into a fluorescence confocal microendoscope will be presented, demonstrating the ability of the system to resolve cellular detail. This achromatized miniature objective allows confocal microendoscopy to use a wide variety of contrast agents while enabling new medical applications in the near infrared range.

8927-10, Session 3

Tethered capsule endomicroscopy with an integrated white light camera for navigation in the upper GI tract

Elena G. Quijano, Michalina J. Gora, Robert W. Carruth, Weina Lu, Drew T. Carlton, Amna R. Soomro, Lorissa A. Moffit, Mireille Rosenberg, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Alessio Fasano M.D., Aubrey J. Katz M.D., Massachusetts General Hospital (United States); Norman S. Nishioka M.D., Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Guillermo J. Tearney M.D., Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and Harvard Medical School (United States) and Harvard-MIT Health Sciences and Technology (United States)

Celiac disease (CD) is an autoimmune disorder of the small intestine that afflicts roughly 1% of the population of the United States. Traditionally diagnosed by endoscopic biopsy, CD is often missed due to inadequate sampling. The risk of obtaining false negatives, along with the substantial cost of the procedure, make endoscopic biopsy an imperfect tool for CD diagnosis. We have developed a swallowable tethered capsule endomicroscope (TCE) that utilizes optical frequency domain imaging (OFDI) to image the entire esophagus at the microscopic level. If properly configured to access the small intestine, this technology could also have the potential to diagnose CD in a minimally invasive, cost-effective, and accurate manner.

The second-generation capsule designed for imaging the small intestine incorporates a white light camera in the distal tip of the capsule for navigation through the stomach. Small wires embedded along the tether deliver power to the camera and transmit video, which is displayed in real time together with OFDI cross-sectional images of the gastrointestinal (GI) tract. The tether is also configured to have mechanical characteristics that aid in maneuvering the capsule through the pylorus of the stomach to the duodenum.

We have successfully tested our second generation TCE for imaging the upper GI tract, including the duodenum and stomach, in swine studies in vivo. We are currently preparing a pilot human study to assess the viability of our TCE device for the diagnosis of CD. Hopefully, our new video-enabled TCE device will not only prove to be a practicable means for CD diagnosis, but will also provide a useful secondary tool for diagnosing BE and other disorders of the upper GI tract through the combination of OFDI and video imaging.

8927-11, Session 3

Tethered capsule endomicroscopy for image-guided biopsy in surveillance of Barrett's esophagus progression

Michalina J. Gora, Harvard Medical School (United States) and Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Amna R. Soomro, Wellman Ctr. for Photomedicine (United States); Weina Lu, Robert W. Carruth, Drew T. Carlton, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Lorissa A. Moffitt, Wellman Ctr. for Photomedicine (United States); Mierille Rosenberg, William Puricelli, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Norman S. Nishioka M.D., Guillermo J. Tearney M.D., Harvard Medical School (United States) and Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States)

Endoscopic biopsy surveillance, the follow-up procedure for patients found to have Barrett's Esophagus, is subject to significant limitations. Since dysplasia and adenocarcinoma are not evident by endoscopy and are focally distributed, random biopsy is inherently prone to sampling errors. To date, we have used a balloon-centering catheter combined with sedated endoscopy to guide esophageal biopsy using OCT images in patients. However, requirement of endoscopy for the balloon procedure increases its time and cost.

We have conceived of a new technology, termed tethered capsule endomicroscopy (TCE) that implements in-vivo microscopy in a swallowable capsule format. We have combined this new catheter with the OCT-guided biopsy platform, in which superficial cautery marks in the esophagus are generated using an additional high-power laser, at sites selected in real-time from OCT images.

In this abstract, we report our results with laser-marking guided biopsy through a swallowable TCE device. The device was first tested in swine in-vivo to determine safety and feasibility of capsule-based laser cautery marking. We tested the following laser parameters: 1450nm tissue irradiation, 400mW power at the tissue, and 1-4 seconds laser pulse lengths in 8 different locations for each pulse length. All cuatery marks were visible with endoscopy and OCT. Histological (NTBC) assessment of tissue showed that damage caused by laser cautery through the capsule was limited to the superficial portions of the esophageal wall. These results and our previous clinical experience indicate that 2 seconds pulse length will be optimal from visibility and safety point of view. We have also confirmed in ex-vivo tissue experiment that marks will be still visible in case of loss of contact with the esophagus. We are currently in the process of beginning human pilot studies with this technique and will report on our initial human experience at the time of the presentation.

8927-12, Session 3

Linear scanning micro-optical coherence tomography probe for imaging of mucociliary transport in airways

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Many pulmonary diseases, including cystic fibrosis (CF) lung disease, are characterized by the failure of mucociliary transport. In healthy airways, mucus acts as a defensive barrier, trapping debris and bacteria, while the cilia lining the epithelial layer transports the mucus towards the pharynx

and out of the airways. In CF, mucociliary transport is known to be defective, though the mechanisms by which it contributes to lung disease remains unknown due to the lack of technology capable of interrogating the airway epithelium at a microscopic scale. Elevated mucus viscosity, abnormal airway liquid volume and ion concentrations, and infection are other factors that may influence lung function deterioration.

Micro-optical coherence tomography (?OCT) is an imaging technique developed in our laboratory that provides 2 ?m lateral resolution and 1 ?m axial resolution, and has been demonstrated as a benchtop platform for imaging mucociliary transport in vitro and ex vivo on airway epithelia. From a single image sequence and without the use of exogenous dyes, several quantitative metrics of airway function can be extracted. Most relevant to CF, these metrics include airway surface liquid (ASL) and periciliary liquid (PCL) thickness, ciliary beat frequency (CBF), and mucociliary transport (MCT) rate.

We now demonstrate micro-optical coherence tomography (?OCT) probes capable of delivering 2 ?m resolution optical performance via a linearly scanned gradient-index (GRIN) fiber endoscope. Depth of focus is extended to ~400 ?m through an annular apodization of the pupil. We present new fabrication techniques for generating the apodized beam geometry, a piezoelectric actuated rigid upper airway ?OCT probe, and a flexible ?OCT bronchoscope. Such technology could provide the first ever imaging of the airway surface capable of discerning the functional microanatomy of the CF and normal lungs.

8927-13, Session 3

Forward-viewing endoscopic OCT catheter using asymmetrically resonant fiber scanner

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This work presents a forward viewing endoscopic OCT catheter based on a resonant fiber scanning. Two-dimensional Lissajous pattern was realized by a piezoelectric tube with quartered electrodes and asymmetrically resonant fiber cantilever. In particular, an additional fiber fragment of 13 mm was attached on a light delivering fiber of 20 mm by using silicon supporting structures and UV curable epoxy. A series of silicon mass was also attached at the proximal end of a fiber cantilever. The resonance frequency of the 20 mm long fiber cantilever was 253 Hz for both x and y directions. Ellipsoidal scan pattern during the 1D operation was observed due to the eccentricity of the PZT tube. The eccentricity generates additional shear forces and induces the mechanical coupling. This seemingly small coupling is in fact significant, when considering the mechanical gain of the resonant operation, since the fiber cantilever has identical resonance frequencies for both axes. However, the additional fiber fragment eliminates the coupling phenomena and clear line scan patterns were obtained by distinguish the resonance frequencies. The resonance frequency of the asymmetric fiber cantilever was 90 Hz and 105.5 Hz for x and y direction. Asymmetrically resonant fiber scanner was assembled with endoscopic housing of 3.2 mm in diameter and combined with SD-OCT system. 3D SD-OCT imaging was successfully demonstrated by implementing Lissajous scanning based 3D image reconstruction algorithm. The endoscopic catheter can be adapted on one of the biopsy channels of conventional endoscope and can provide a new direction for endoscopic diagnosis.

8927-14, Session 3

Improved spatial frequency response in optical coherence tomography imaging using a depth-encoded pupil mask

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The availability of ultra-broadband light sources has pushed the axial resolution of optical coherence tomography (OCT) imaging to the micron level. The transverse resolution of an OCT system, however, suffers from a trade-off with depth of focus. A high numerical aperture (NA) can generate micron-level lateral resolution, but also reduces the depth of focus. Beam geometries such as Bessel beam illumination which maintain tightly confined central lobe size over a large distance have been used to increase the depth of focus. However, Bessel beams suffer from strong side-lobe artifacts because of a mid-range frequency gap in the coherent transfer function (CTF), though higher spatial frequencies are accessible to Bessel beams when compared to Gaussian beam geometry.

We now demonstrate a technique that combines multiple simultaneous pupil geometries wherein the CTF of each beam pupil segment contains gaps in spatial frequency support, but can be combined in a complementary fashion to generate a total CTF with a broad and flat passband. The pupil is divided into zones by a phase mask with a unique optical path length for each zone. The beam resulting from each pupil zone represents a range of spatial frequencies, independently encoded by optical delay. The sub-images arising from combinations of illumination and detection aperture zones are thus separated in depth on a single resulting OCT image. The CTF from each pupil segment can be measured, then coherently filtered and summed with the filtered sub-image from each other combination of apertures such that the total image achieves broad spatial frequency support and is smooth in both magnitude and phase throughout the passband, mitigating side-lobe artifacts while maintaining high resolution over a broad range of depths.

In a proof-of-concept configuration, a two-zone pupil mask with a thick-glass circular inner zone and a thin-glass annular outer zone was utilized, creating three OCT sub-images corresponding to outer-outer, inner-inner and outer-inner illumination and detection aperture combinations, each acquired simultaneously at differing apparent depths in a single OCT image. The three images respectively contribute high, low and intermediate frequencies and are combined through coherent filtering to generate improved total frequency response. This configuration is adaptable to an endoscopic probe in which a partially drawn fiber preform serves as the phase mask, with the index of refraction mismatch of the core and cladding material creating the depth-encoding path difference.

8927-32, Session PSun

GRIN lens-based endoscopic micro-optical coherence tomography

JunYoung Kim, Minwoo Lee, Hongki Yoo, Hanyang Univ. (Korea, Republic of)

Optical coherence tomography (OCT) acquires three-dimensional microstructure of biological samples. In general, it has ~10 μm resolution in both axial and transverse directions, which is not enough to visualize individual cells and sub-cellular structures. Micro-optical coherence tomography (μ-OCT) has improved spatial resolution up to 1 μm, enabling clear visualization of cellular and sub-cellular features of biological samples. Up to our knowledge, endoscopic imaging probe of μ-OCT has not yet been developed. Since miniaturized endoscopic imaging probe or imaging catheter is essential for investigating internal organ in vivo, we have developed endoscopic micro-optical coherence tomography using gradient index (GRIN) lens and apodization. We used GRIN lens due to its small size (1.0 mm diameter) and converging capability. The forward-looking imaging probe has a outer diameter of 1.0 mm and a length of 9.7 mm. In order to increase the depth imaging range, we applied apodization technique. Simulation analysis using ZEMAX shows that the designed GRIN lens probe has a spot radius of 2.08 μm and an imaging range of 89 μm. By applying apodization technique, we acquired 1.5-fold increase of imaging range with slightly smaller spot size. The performance of the developed μ-OCT endoscopic GRIN imaging probe was tested using a phantom made of agarose gel with micro-beads (1 μm). Experimental results showed the extended depth of focus with high spatial resolution. We anticipate that this high-resolution imaging probe might be useful for various in vivo studies.

8927-15, Session 4

Image quantification for widefield molecular endoscopy to detect esophageal neoplasia

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Esophageal adenocarcinoma is rising rapidly in incidence. Premalignant lesions (dysplasia) are challenging to detect on conventional endoscopy because of their flat appearance. Peptides are promising for use as specific targeting agents because of their high clonal diversity, small size, and broad compatibility with fluorescence dyes, and rapid binding kinetics. We aim to quantify the fluorescence intensities from peptide binding to esophageal neoplasia in vivo on wide-field endoscopy. Wide field imaging using a fluorescence endoscope (excitation wavelength=450-490 nm) was performed in n=54 human subjects after topical administration of a FITC-labeled peptide. Visible fluorescence (emission wavelength=510-650 nm) and reflectance images (reflectance wavelength=540-560 nm) were collected consecutively in vivo. An image quantification technique is developed by using the reflectance image to correct for differences in distance and geometry of the fluorescence images. The quantification technique is validated by imaging a fluorescent phantom at different distances and angles. The same algorithm is applied to in vivo images. Preliminary results showed that the image quantification technique can be used to correct for geometric differences. The endoscopic appearance of the lesions found in n=54 patients were identified per Paris classification. Fluorescence was collected from the surface epithelium of squamous, Barrett's esophagus, low-grade dysplasia, high-grade dysplasia, and esophageal adenocarcinoma. We measured a SNR of 5.9±4.1 and a TBR of 8.5±5.8 from the target region at a threshold of 40. We have quantified in vivo fluorescence intensities from specific peptide binding to esophageal neoplasia. This integrated imaging strategy has potential to be a "red flag" technology to guide tissue biopsy.

8927-16, Session 4

Diffuse optical spectroscopy probe for therapy monitoring in colorectal cancer

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Rectal cancer is characterized by a low 5-year survival rate. To increase survival, typically neoadjuvant chemotherapy and radiation therapy are combined and given. However, very often (up to 30%) such therapies do not reach the desired pathological complete response. Therefore the need of monitoring tumor response arises. Diffuse optical spectroscopy imaging (DOSI) has previously been successful in the prediction of neoadjuvant chemotherapy response in breast cancer, as early as one day after the beginning of the therapy. Patients with rectal cancer receive similar chemotherapy treatments. Measurements in the rectum pose multiple challenges and constraints to conventional DOSI instruments. The rectum geometry, healthy and tumor tissue properties in the rectum and the requirement of surface contact impose constraints on the probe design. Optical properties of deeper tissue in the rectum are uncertain and may vary, therefore measurement uncertainties are increased. No direct vision is available to the probe operator and the probe must be safe for internal use and easy to clean. In this work we present a design of a DOSI probe with the aim of early chemotherapy/radiotherapy effectiveness detection in rectal tumors. Monte Carlo simulations and phantom measurements have been used to show that colon tissue can be characterized reliably using a source-detector separation in the order of 10 mm. We present a design and rapid prototype of a probe for DOSI measurements that can be mounted on a standard laparoscope and that fits through a standard rectoscope. Using predominantly clinically approved components we aim at fast clinical translation.

8927-17, Session 4

Clinical translation of real-time color and near-infrared fluorescence endoscopy: feasibility study in cholangiopancreatoscopy

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The diagnosis of malignancies via endoscopy remains an important challenge in contemporary clinical practice. White light endoscopic imaging is widely available and routinely used for disease inspection, however, its sensitivity for tumor identification and demarcation has been reported as insufficient due to the limited ability of the human eye to visualize below the surface and to differentiate tissue on a molecular level. The recent clinical propagation of targeted fluorescence agents brings a promising new alternative to endoscopy by complementing visual disease detection with invisible physiological and molecular biomarkers.

In this work we developed real-time near-infrared (NIR) cholangioscopy and pancreatoscopy, characterize the imaging system and validated its clinical use. A spatial optical resolution of about 50 ?m was achieved and fluorescent dye concentrations of 17.3 nM, corresponding to molecule amounts in the femtomole range, could be detected. We demonstrate the clinical feasibility of real-time wide-field color and NIR fluorescence endoscopy in detecting indocyanine green (ICG) localization in pancreatic cancer and cholangiocarcinoma during surveillance cholangiopancreatoscopy in two patients. Maligancy was confirmed by tissue biopsies. The results demonstrate the feasibility of wide-field NIR fluorescence imaging through adapted endoscopes. The advent of targeted molecular fluorescent agents could enable a red-flag detection strategy for cancer screening.

8927-18, Session 4

Multispectral scanning fiber endoscope with concurrent autofluorescence background mitigation for improved target-to-background ratio

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Background: Fluorescent molecular probes targeting disease specific biomarkers are under development for clinical applications. In particular, detection of early cancerous lesions using molecular imaging can improve early disease diagnosis and increase survival. The challenge for the next generation of endoscopic molecular imaging technology is providing sufficient image contrast for small pre-cancerous lesions in narrow lumens and ducts in vivo. Also needed are quantitative fluorescence imaging with red-flagging and ranking for biopsies. However, tissue autofluorescence (AF) can limit the ability for quantitative

fluorescence imaging. To address this limitation a wide-field multi-spectral scanning fiber endoscope (SFE) was developed, with the capability to increase contrast of fluorescent images by mitigating background AF.

Methods: A realistic 3-dimentional tissue phantom was fabricated to mimic both healthy and early-cancerous surface lesions with an AF background. Selectively filtered PMTs in the SFE system separately recorded the phantom biomarker probes labelled fluorescence and the background autofluorescence. A 488 nm diode laser was used to excite the dye fluorescence and another laser (448 nm) only excited the AF. Fluorescence contrast enhancement with simultaneous AF background subtraction was demonstrated for FITC, an FDA approved dye.

Results: Imaging was performed on fluorescent targets that matched in vivo dye concentration at high resolution (50 um), wide field of view (80º), and at 30 Hz video rates. Target-to-background-ratio of FITC labeled molecular imaging was increased from 2 to 10.

Conclusions: The newly developed multi-spectral fluorescence SFE technology and AF mitigation methodologies have improved the performance of FITC biomarker imaging for upcoming clinical applications.

8927-19, Session 5

Compound vari-focal objective lens for confocal endomicroscopy

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The diagnosis of esophageal diseases such as Barrett's esophagus, eosinophilic esophagitis, dysplasia and intramucosal carcinoma remain important clinical problems.Imaging large areas of the esophagus with microscopic resolution in vivo may offer an improved diagnostic solution compared to the standard of care, random biopsy. Spectrally encoded confocal microscopy (SECM) is an endomicroscopy technology that is capable of imaging large areas of the esophagus by helically scanning the probe optics. One key challenge for large-area SECM imaging of the esophagus is maintaining the same imaging depth within the tissue during the helical scan. This task can be accomplished by incorporating an adaptive focusing mechanism within the SECM probe. In this presentation, we report the development of a miniature vari-focal objective lens that can be used to adaptively change the SECM focal depth. The vari-focal objective lens was composed of an aspheric singlet (focal length = 1.6mm; NA = 0.5w) and a vari-focal liquid lens. In the vari-focal liquid lens, a cylindrical chamber (thickness = 0.8mm) with a thin Polydimethylsiloxane (PDMS) membrane (130?m) was filled with water. Miniature tubings were connected to the cylindrical chamber, and the water pressure was controlled manually by changing the pressure of the fluid. The vari-focal objective lens assembly had a total diameter of 5mm and thickness of 4mm. The focusing range was measured to be 150 ?m. Excised swine esophageal tissues, stained with 6% acetic acid, were imaged at multiple imaging depths using the vari-focal objective lens. Nuclei of the squamous epithelium could be clearly visualized at different depths with the vari-focal lens and SECM optics.

8927-20, Session 5

Double-clad fiber coupler for confocal endomicroscopy at 800 nm

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We report the development of a novel double-clad fiber coupler (DCFC) specifically designed for near infrared confocal microscopy, confocal endomicroscopy and fluorescence imaging. The custom designed double-clad fiber (DCF) supports the propagation of a single mode (cut-off wavelength 610nm) in its 4 micron-diameter core (0.12 NA), while allowing as many as 150 modes in its 25 micron-diameter inner cladding (0.19 NA). The inner-cladding-to-mode-field-diameter ratio of ~6 allows for reduction in speckle contrast, while preserving the optical sectioning of confocal imaging.

A DCFC is obtained using a standard fusion tapering technique and allows for achromatic (from 750 nm to 850 nm) transmission through the core (>90%) and achromatic extraction of >70% of inner cladding multimodal signal. The all-fiber coupler is inserted in a spectrally encoded confocal microscopy system using a simple splice with the laser's SMF750 fiber having similar mode-field-diameter and NA. The laser is a polygon-based wavelength-swept laser centered at 780 nm (bandwith 40nm, sweep rate up to 15kHz, 50mW) and is used in conjunction with a 1800 grooves/mm transmission diffraction grating to provide 512x512 pixels imaging at 30 frames/s. The inner cladding area is used to collect speckle-free reflectance or fluorescence signal (e.g. Alexa Fluor), or both with high signal to background ratio.

8927-21, Session 5

Image-guided therapy by spectrally encoded endoscopy

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Spectrally encoded endoscopy (SEE) is an endoscopic imaging technology that can conduct high-definition video imaging through ultraminiature probes with diameters of less than 1 mm. One of the clinical areas that SEE can be utilized for is image-guided therapy of fetal diseases. SEE probe can be inserted inside the uterus and can image the fetus and placenta to identify regions that cause the fetal diseases. Once the culprit regions are located, high-power therapy laser can be coupled into the SEE probe to coagulate or ablate the diseased sites. In this presentation, we report the first demonstration of image-guided therapy using SEE in a living animal. The SEE probe (diameter = 500 μm) used a dual clad fiber (DCF) to deliver the illumination light (415-700 nm) through the core and the therapy light (532 nm) through the inner cladding. A fiber-based DCF coupler was developed that combined the imaging and therapy light. To demonstrate the capability of the SEE probe to perform image-guided therapy, we used murine peritoneal vessels as an in vivo model. The probe was introduced into the peritoneal cavity through a modified 20Ga needle (outer diameter = 0.9mm) and was maneuvered to locate the target vessel at the center of the image. The vessel was exposed up to 20 sec of the 250mW therapy laser light. SEE movies obtained in vivo allows the ablation of the target vessel to be clearly visualized in real time. The time to reach the coagulation varied depending on the vessel size and the working distance between the SEE probe and tissue.

8927-22, Session 5

Miniature, forward-viewing probe for spectrally encoded endoscopy

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By replacing rapid mechanical scanning with spatial wavelength encoding, spectrally encoded endoscopy (SEE) promises miniature, small diameter endoscopic probes that allow easy access to hard-to-reach locations within the body. Using low numerical aperture imaging and low coherence interferometry, SEE has been shown capable of three-dimensional, subsurface, and Doppler imaging with high signal-to-noise ratios through sub-millimeter endoscopic probes. The side-view of current SEE miniature probes, however, prevents their use in navigating through narrow passages and small-diameter ducts, where such probes could be particularly useful. In this work, we present a new SEE probe design with a forward-viewing angle that is suitable for imaging within small-diameter ducts and vessels, and demonstrate high-quality imaging with a wide field of view, a large number of resolvable points and low speckle noise. Using a separate illumination channel of incoherent broadband light across the visible range, the new probe is capable of color and spectral imaging as it advances through the main axis of the cylindrical duct. Potential applications of the new probe include early detection of malignancies within the mammary ducts and the lungs, and imaging within blood vessel for the detection of various vessel wall pathologies.

8927-23, Session 6

Pulse shaping for optimum pulse delivery through an image guide fiber bundle at 775-nm wavelength

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Nonlinear laser-scanning microscopy is a well established technique and has been used for high-resolution imaging in bio sciences. With the advances in micro-optics and micromechanical components, a nonlinear endomicroscopy system is becoming attractive candidate for a potential in vivo clinical diagnostic tool. Even though a fiber bundles is a standard light delivery and detection tool for endoscopy, it not easy to be used in nonlinear endoscopy because of pulse distortion and nonlinear spectrum broadening in a fiber bundle. In order to avoid this problem, specialty fibers with specific dispersion characteristics were employed for nonlinear endoscopy. In this paper we present a nonlinear endomicroscopy with a fiber bundle with pre-chirped pulse shaping. We have used a stable Er-doped fiber laser as a seeding light source. By using a second harmonic generation (SHG) crystal, we made a 775 nm light source for the two-photon endomicroscopy. 3 m long fiber bundle with 30,000 cores were used. By measuring dispersion of the fiber bundle, we have obtained the optimized the pre-chirping condition with a grating pair just before a pulse is launched to the fiber bundle. We present the feasibility of a fiber bundle based nonlinear fluorescence endomicroscopy with optimized pre-chirped pulse condition. Since there is no mechanical scanning part in our system, we believe that it is potentially more robust and practical for possible clinical applications compared to a single fiber based head-scanning endomicroscopy.

8927-25, Session 6

Fiber-optic endomicroscopic two-photon fluorescence lifetime imaging

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Fluorescence lifetime imaging (FLIM) has been shown as a useful quantitative method for cancer detection based on the change in fluorescence lifetime of endogenous fluorophores (such as NADH and FAD) or exogenous fluorophores (such as 5-ALA-induced PpIX). Clinical application of FLIM for cancer imaging in internal organs requires an endoscopic instrument. Currently prevalent implementations of the endoscopic FLIM utilize single-photon excitation and an imaging fiber bundle, which often requires a confocal configuration and leads to a complicated endoscope setup, sub-optimal resolution and low photon detection efficiency. Other implementations often employ time-gated fluorescence detection for lifetime determination, which eliminates many photons. Recently scanning fiber-optic endomicroscopy technology has been developed for performing two-photon fluorescence imaging; yet FLIM has not been demonstrated with a scanning fiber-optic endomicroscope. Here we report an endomicroscopic FLIM system which integrates two-photon excitation and time-correlated single-photon counting (TCSPC) detection. The endomicroscope can deliver the excitation light, scan the imaging beam, and collect the emission light. As in a laser scanning microscope, two-photon excitation offers intrinsic optical sectioning capability and thus superb spatial resolution. Different from time-gating, TCSPC makes use of almost every emission photon, achieving a high signal-to-noise ratio. Initial experimental results with stained cell culture and histological tissue slides have demonstrated that our system can accurately evaluate fluorescence lifetime with high spatial resolution and a moderate frame rate. Further tests on unstained breast and brain cancer tissues are underway, and the potential of an endomicroscopic FLIM system for cancer detection will be assessed and discussed.

8927-26, Session 6

Development of a miniaturized side-looking probe based two-photon microscopy and optical coherence tomography

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Colorectal cancer is usually screened by wide-field colonoscopy. Because colonoscopy can examine surface of the folded colon only, there is a relative high missing-rate of detection. There have been various efforts in development to reduce the missing-rate, such as molecular imaging probes targeting specific markers, high-resolution imaging probe to examine at cellular levels, and 3D imaging techniques to examine tissue structures. We developed a miniaturized rigid probe based two-photon microscopy (TPM) and optical coherence tomography (OCT). This system is to study the colorectal cancer in animal models, in vivo.

TPM and OCT was to examine cellular structures at local regions with and without fluorescent markers, and cross-sectional tissue structures in large sections respectively. The probe consisted of gradient index (GRIN) lenses and a reflecting prism at its distal end, and it was attached in front of an objective lens based typical microscopic system. This imaging probe was 2.2 mm in diameter including a protecting sleeve. TPM images were acquired by high-speed raster scanning at 15.4 frames/s, and its field of view was 250 ?m ? 250 ?m in the x-y plane. OCT images were acquired by translating tissue samples along the probe for large-sectional imaging, and its imaging speed was 40 mm/s at one cross-section. We characterized the endoscopic imaging probe system with a tissue phantom containing microspheres, and applied to image the mouse colon tissue samples. We can see a gland cellular morphology and flat layer structures in normal colon, but we can visualize a distorted and thicker gland structure in colorectal cancer model, ex vivo. We'll try to demonstrate these phenomena using AOM/DSS treated mouse models, in vivo.

8927-27, Session 7

Fluorescence microendoscopic system for imaged-guided holographic photostimulation and laser microsurgery

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Innovations in optical microscopy have successfully been brought, together with the advanced fiber-optic and miniaturized optics technologies, into the realm of minimally invasive in vivo diagnostics with cellular resolution. The main thrust in microendoscopy is to miniaturize the probe in order to ensure flexible maneuver during imaging within the hard-to-reach and complex internal organs, especially brain and small lumens. Equally important is to enable the same endoscopic system for prompt, if not simultaneous, precise intervention on the same field-of-view. Such capability aligns well with the spurred interest in study of neuronal activities using photostimulation or optogenetics, and the pressing needs for high-precision image-guided laser microsurgery. However, realizing precise spatiotemporal stimulation of neuronal structures in deep brain tissue (>5-6 mm) remains non-trivial. Techniques of generating complex spatiotemporal laser surgical patterns in vivo also remain rather under-explored. To this end, we developed a compact and flexible endoscopic system which can deliver a user-defined holographic illumination patterns with cellular resolution (~1-3 ?m) and milliseconds temporal resolution (5.5 ms), based on a spatial light modulator (SLM), through an fiber bundle compatible with a 25-gauge needle. The system can simultaneously perform high-resolution fluorescence microscopy through the same fiber bundle – a crucial feature maintaining the system compactness and flexibility. We here report the details of the fiber-bundle system design and the preliminary demonstration of the patterned illumination for photostimulation, simultaneously with fluorescence microendoscopy. We also discuss strategies to implement deep-brain holographic photostimulation and image-guided holographic laser microsurgery based on our current system.

8927-28, Session 7

Volume holographic reflection endoscope for in-vivo ovarian cancer clinical studies

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We present the design for an endoscopic system capable of imaging tissues of the ovary at two selected imaging depths simultaneously. The method utilizes Volume Holographic Imaging capabilities developed by the group and previously used in an ex-vivo clinical study. The design utilizes both gradient index (GRIN) optical components and off the shelf singlet lenses to relay an image from the distal tip to the proximal end. The endoscope has a maximum diameter of 3.75 mm at the proximal end. The system length is 30 cm which is connected to a handle that includes the holographic components and optics that relays the image to the CCD. Preliminary evaluation of the endoscope was performed with reflection tissue phantoms and calibrated targets and shows lateral resolution < 4 ?m at an operating wavelength of 660 nm. The hologram is recorded in phenanthraquinone doped poly methacrylate and is designed to produce images from two tissue depths. One image is obtained at the tissue surface and the second 70 µm below the surface. This method requires no mechanical scanning and acquires an image at the camera frame rate. The preliminary ex-vivo results shows good correlation with histology sections of the same tissue sections.

8927-29, Session 7

The potential for store bought foods/liquids to provide oral contrast for reflectance confocal microscopy of the esophagus

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Esophageal confocal laser endomicroscopy (CLE) commonly utilizes i.v. fluorescent agents to provide nuclear image contrast. The use of these exogenous agents increases the cost and complexity of the imaging procedure. Reflectance confocal microscopy, which detects light scattering as opposed to fluorescence, has the advantage that it does not require the administration of i.v. agents, but suffers from relatively poor image contrast. Acidic solutions such as acetic acid have been used to provide improved nuclear contrast for reflectance imaging, but must be applied topically using an endoscope. In this abstract, we describe a study that tests the capability of different commercially available foods/liquids to provide nuclear contrast for reflectance confocal microscopy, with the goal of providing a safe and tolerable, swallowable formulation for enhancing contrast in reflectance confocal microscopy of the esophagus.

We tested a variety of store-bought foods/liquids on freshly excised and opened swine small intestine (a proxy for Barrett's esophagus). The list of foods/liquids included different vinegars, fruit juice, and sour candies, such as apple drinking vinegar, 100% lemon juice, and Sour Patch Kids. The substances were instilled or placed on the top of the small intestine lumen for the liquids and solids with 1 minute. Imaging was conducted using spectrally encoded confocal microscopy (SECM), a high-speed form of reflectance confocal microscopy.

Many store bought foods/liquids provide good contrast for reflectance confocal microscopy. Future studies will be conducted to assess the performance of these substances in vivo and to evaluate the tolerability of swallowing these different foods/liquids.

8927-30, Session 7

Ultrahigh speed endoscopic swept source optical coherence tomography using a VCSEL light source and micromotor catheter

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We developed an ultrahigh speed endoscopic swept source optical coherence tomography (OCT) system for clinical gastroenterology using a vertical-cavity surface-emitting laser (VCSEL) and micromotor based imaging catheter, which provided an imaging speed of 600 kHz axial scan rate and 8 um axial resolution in tissue. The micromotor catheter was 3.2 mm in diameter and could be introduced through the 3.7 mm accessory port of an endoscope. Imaging was performed at 400 frames per second with an 8 um spot size using a pullback to generate volumetric data over 16 mm with a pixel spacing of 5 um in the longitudinal direction. Three-dimensional OCT (3D-OCT) imaging was performed in patients with a cross section of pathologies undergoing standard upper and lower endoscopy at the Veterans Affairs Boston Healthcare System (VABHS). Patients with Barrett's esophagus, dysplasia, and inflammatory bowel disease were imaged. The use of distally actuated imaging catheters allowed OCT imaging with more flexibility such as volumetric imaging in the terminal ileum and the assessment of the hiatal hernia using retroflex imaging. The high rotational stability of the micromotor enabled 3D volumetric imaging with micron scale volumetric accuracy for both en face and cross-sectional imaging. The ability to perform 3D OCT imaging in the GI tract with microscopic accuracy should enable a wide range of studies to investigate the ability of OCT to detect pathology as well as assess treatment response.

8927-31, Session 8

Vertical cross-sectional imaging by multi-spectral handheld dual axes confocal endomicroscope

Zhen Qiu, Haijun Li, Xiyu Duan, Supang Khondee, Bishnu Joshi, Kenn R. Oldham, Katsuo Kurabayashi, Thomas D. Wang M.D., Univ. of Michigan (United States)

We have demonstrated vertical cross-sectional imaging with a near-infrared (NIR) multi-spectral (671 and 785nm) dual axes confocal fluorescence endomicroscope. This 3-dimensional (3D) handheld imaging instrument uses a novel 3D scanning mechanism that includes a 2-dimensional (2D) micro-mirror consisting of a resonant in-plane comb-drive configuration MEMS scanner and z-axis actuation with a translational piezoelectric micro-motor. The 2D resonant device, fabricated with a 4-mask SOI MEMS process, utilizes a parametric resonance mechanism. With low drivevoltage, this scanner can achievea large scanning angle (±6 deg mechanical) with a 200Hz tunable frequency bandwidth close to resonance at ~3kHz. The dumb-bell shaped mirror is 2.71 mm in length and 650 um in width, and the surface is coated with Au/Ti to achieve optical reflectivity >90% from 671-785nm. This compact NIR fluorescence imaging instrument has an outer diameter of 5 mm and a length of 25 mm at the distal end, and is fully packaged and sealed. A large field-of-view (800 um by 400 um) can be achieved in the XZ-plane with 5 um axial resolution and deep tissue penetration at 5 Hz frame rate. To characterize vertical cross-sectional imaging, we collect images from a 3D bead phantom with Cy5.5 (671nm) staining. Ex-vivo multi-spectral vertical cross-sectional imaging on colorectal cancer mice model has been demonstrated with Cy5.5 labeled specific binding peptide and Li-COR dye (785nm) staining. The XZ view shows the relationship among tissue micro-structures as they vary with depth. This instrument can achieve real time histology-like imaging in vivo in the preferred view of pathologists.

Conference 8927B: Optical Techniques in Pulmonary Medicine

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8927 Endoscopic Microscopy IX; and Optical Techniques in Pulmonary Medicine

8927-33, Session 9

Bronchoscopy in asthma, COPD and lung transplant *(Invited Paper)*

Kyle Hogarth M.D., Univ. of Chicago Medicine (United States)

No Abstract Available

8927-34, Session 9

Co-registered optical coherence tomography and autofluorescence imaging of human lung

Hamid Pahlevaninezhad, Anthony M. Lee, Rashika Raizada, Lucas Cahill, Stephen Lam, Calum MacAulay, Pierre M. Lane, The BC Cancer Agency Research Ctr. (Canada)

Optical coherence tomography (OCT) is a powerful technique for imaging sub-surface tissue morphology and structure. Autofluorescence (AF) imaging of endogenous tissue fluorophores provides valuable information about the biochemical composition and metabolic state of the tissue. Although AF can be detected at the surface of the tissue, the AF signal associated with different components cannot be accurately determined due to the absorption and scattering of light by different tissue layers. By co-registering OCT and AF, the structure and function of tissue structures can be examined. In this pilot study, we develop a fiber-based AF-OCT system for imaging human airways in vivo with the goal of studying airway diseases such as asthma, chronic obstructive pulmonary disease, and lung cancer. The AF and OCT light path are combined in a custom-made fiber-optic rotary joint unit. A common-path optical fiber probe based on a double-clad fiber capable of operating at both OCT and AF wavelengths allows for co-registered AF-OCT imaging. We tested the system in human bronchi ex-vivo. Cartilage and dense connective tissues were found to be dominant fluorescing components and the epithelium and loose connective tissue near basement membrane generate very small AF signal. The in vivo results will be presented.

8927-35, Session 9

Optical coherence tomography (OCT) for management of major airway stenosis

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Stenosis of major airways occurs in a number of benign pulmonary diseases such as vasculitis, endobronchial tuberculosis, and post lung transplantation. Currently, high resolution CT scan, and visual examination via bronchoscopy are used to determine the severity of the stenosis. However, these only give a rough estimated measurement of airway stenosis. We hypothesize that Optical Coherence Tomography (OCT) imaging via flexible bronchoscopy allows more precise measurement of airway size and structure to guide choice of therapy.

Method: OCT imaging was performed in 7 main airways stenosis in three symptomatic patients with underlying vasculitis who underwent bronchoscopy for therapeutic airway balloon dilation. OCT imaging was done pre- and post dilatation at the same airway segments by inserting a 1.5 mm diameter probe through the stenotic region. 3-D OCT imaging of 5 cm long airway segments was performed by the Lightlabs C7XR system (St. Jude Medical, Inc., St. Paul, MN, USA).

Result: A total of 11 studies were performed. OCT imaging was found to be useful in assessing the integrity of the cartilage, presence or absence of inflammation, degree of fibrosis in the non-cartilaginous region of the airways and for determining the balloon size for dilatation. Changes in the cross sectional area of the stenotic segments pre- and post balloon dilation correlated with the clinical outcome.

Conclusion: OCT imaging via flexible bronchoscopy is a safe and accurate method to measure airway dimension and structure as well as changes after balloon dilation. These measurements can be used for therapeutic guidance and follow up.

8927-36, Session 9

Evaluation of airway response to segmental allergen challenge by optical frequency domain imaging

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The objective of this study was to use optical frequency domain imaging (OFDI) to assess morphological differences in airway structure between healthy and asthmatic volunteers at baseline and 24 hours after titrated allergen exposure. Methods: The right middle lobe and airway control areas were imaged in 8 mild allergic asthmatic and 15 allergic non-asthmatic volunteers. Baseline images and lung lavages were collected on day 1, and topical allergen was placed in the right middle lobe. Images and lung lavages were collected again 24 hours later. OFDI images were analyzed, and measurements of epithelial thickness and mucosal thickness normalized by airway perimeter (MT/P) were used to assess airway morphology and allergic response. Bronchoalveolar lavages provided data on airway eosinophil, neutrophil, and lymphocyte counts. Results: At baseline, both the normalized mucosal thickness and epithelial thickness were significantly larger in allergic asthmatics. There was no significant difference between baseline eosinophil counts between groups. Both groups exhibited an increase in mucosal thickness after allergen exposure, but this increase was significant only in allergic non-asthmatics. Post-allergen, there was no significant difference between MT/P between groups. Eosinophil counts increased significantly after allergen administration in both groups. Additionally, we found that raw change in eosinophil counts in the allergen challenged airway was positively correlated with the change in normalized mucosal thickness in allergic asthmatics, but not in allergic non-asthmatics. Conclusion: Baseline differences between groups may indicate airway remodeling. OFDI is a non-invasive, practical tool for assessing lung morphology in asthmatics.

8927-37, Session 9

Sex differences in chronic obstructive pulmonary disease evaluated using optical coherence tomography

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Research Ctr. (Canada); Peter K. Laratta, Don D. Sin, St. Paul's Hospital (Canada); Annette M. McWilliams, Stephen Lam, The BC Cancer Agency Research Ctr. (Canada); Harvey O. Coxson, The Univ. of British Columbia (Canada) and St. Paul's Hospital (Canada)

Rationale: Sex differences are being increasingly recognized in patients with chronic obstructive pulmonary disease (COPD). Optical coherence tomography (OCT) is a technique capable of imaging small bronchioles with resolution approaching histology making it an ideal procedure to study sex differences in airway wall structure. Our hypothesis was that small airway wall dimensions measured using OCT images are associated with airflow limitation in COPD and is different in males and females.

Methods: Eight-six current or ex-smokers (n=43 males, n=43 females) were enrolled in the BC Lung Health Cohort and underwent OCT imaging and spirometry. OCT imaging was performed using a 1.5mm diameter probe in either the right lower lobe (RB8 or RB9) or the left lower lobe (LB8 or LB9). Measurements of small airway lumen area, wall area and the percentage of the airway that is wall (wall area percent, WA%) were obtained by tracing the inner and outer boundary of the airway wall using ImageJ software (NIH, USA).

Results: There were no significant differences between males and females for FEV1 ($p=0.14$) or WA% ($p=0.14$). However, the WA% was greater for females than males in subjects with no or minimal airflow obstruction ($p=0.002$ and $p=0.04$, respectively), but not in more severely obstructed subjects ($p>0.17$). There was a significant correlation between WA% and FEV1/FVC ($r=-0.60$, $p<0.0001$) for males, but not for females ($r=-0.24$, $p=0.12$).

Conclusions: These data suggest that there are differences in airway wall dimensions in male and female smokers and these differences change with disease progression.

8927-38, Session 10

Real-time assessment of dynamics in lower airway using high speed endoscopic OCT

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Obstructive lung diseases (OLDs) are a category of serious respiratory diseases characterized by airflow limitation often associated with pathology in the conducting airways of the lungs, including asthma, emphysema, and chronic bronchitis. The well-known morphological changes in OLDs include increases in airway wall thickness, often associated with increased airway smooth muscle contraction. Quantitative analysis of these structural changes in airways plays an important role in understanding the pathogenesis of the OLD. In this study, we developed a high-speed endoscopic swept-source optical coherence tomography (SS-OCT) system, including a 40-220 kHz A-scan FDML laser, a miniature (1.3 mm in diameter) side-viewing OCT catheter and a software platform enabling real-time data acquisition, processing and saving. A canine model was used to compare in vivo OCT images with the corresponding airway histology. , Initial results showed excellent correlations between the OCT images and post-mortem histology. . The same imaging system was then employed in a swine model to dynamically assess structural changes of the lower airways after methacholine challenge, which mimicked the constriction seen in asthma. Changes of luminal area and wall thickness were clearly identified on OCT images in real time and quantitatively analyzed. Preliminary quantitative results showed that the luminal area of the airway was reduced by more than 40% while the average wall thickness of the airways was increased by about 10% with methacholine challenge. These results are consistent with the expected changes in anatomic models where the wall mass does not acutely change with smooth muscle contraction.

8927-39, Session 10

Smoke inhalation injury on a sheep animal model using long range optical coherence tomography

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The ability to detect airway mucosal and submucosal injury is valuable for assessment of smoke inhalation injury. Optical coherence tomography (OCT) has the potential ability to monitor the progression of airway injury changes such as edema and swelling, which are critical clinical components of smoke inhalation injury. Previous studies have shown that OCT can be used to detect significant smoke-injury-induced increases in the thickness of the airway walls of rabbits beginning shortly after smoke inhalation. However, a conventional OCT system does not have the required imaging range to fully cover larger diameter airway of an adult human or a larger animal model. Therefore, we utilized a long range OCT system with ~20mm imaging range to investigate morphological airway changes following smoke inhalation injury in a sheep model. The animal subject was monitored every 24 hours for 5 days starting just before and almost immediately after smoke inhalation injury using an OCT probe with 1.2mm OD and 15mm working distance. Cross sectional images of the airway were obtained at 25 frames per second during each pullback of 20cm, covering from the right mainstem just below the carina and most of the trachea. Long range OCT was able to fully image the airway and detect significant changes of the airway walls. Thickness of mucosal and submucosal layers were measured similar to that in previous studies, showing progression of injury and recovery of the airway tissue.

8927-40, Session 10

Optical monitoring of physiologic and metabolic effects of sodium sulfide (NaSH) poisoning and treatment with hydroxocobalamin and cobinamide

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Background and Purpose: Hydrogen sulfide (H2S) is a highly toxic gas for which no effective antidotes exist.. It acts, at least in part, by binding to cytochrome c oxidase, causing cellular asphyxiation and anoxia.

We used diffuse optical spectroscopy (DOS) to detect sulfide-induced toxicity and monitor the effects of two novel antidotes—hydroxocobalamin and the cobalamin analog cobinamide.

Methods: 24 New Zealand white rabbits were divided into five groups: intravenous (IV) saline (control group), IV hydroxocobalamin, IV aquohydroxocobinamide, IV sulfitocobinamide, and intramuscular (IM) sulfitocobinamide. NaSH was infused intravenously, blood gasses were sampled, and deoxygenated and oxygenated hemoglobin were measured

continuously in the brain and muscle by DOS and continuous wave near infrared spectroscopy (CWNIRS).

Results: CWNIRS of the brain showed an initial decrease in oxyhemoglobin and concomitant increase in deoxyhemoglobin followed by a reversal of the changes and increase in total hemoglobin as poisoning progressed. The initial change correlated with a decrease in blood pressure, leading to decreased tissue perfusion as observed by lower tissue hemoglobin levels. Animals in all three cobinamide groups and the cobalamin group showed reversal of the changes in hemoglobin oxygenation and concentrations, and tolerated significantly more NaSH than control animals, with the animals in the IV aquohydroxocobinamide and sulfitocobinamide groups tolerating much more NaSH than the IV cobalamin group.

Conclusions: DOS monitoring demonstrated that cobinamide is an effective agent for reversing lethal sulfide exposure.

8927-41, Session 10

Electromagnetic optical coherence tomography for assessment of the pulmonary airways

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Lung cancer is a leading cancer of cancer death. Accurate and early stage diagnosis of lung cancer is critical for increased survival rates. Macroscopic CT imaging is highly sensitive for detecting solitary pulmonary nodules (SPNs) that may be cancerous, however, it does not have sufficient resolution to diagnose malignancy. We have previously demonstrated that OCT can be used to assess pulmonary pathology relevant to lung cancer. We have developed a novel high-resolution multimodality imaging platform to assess SPNs both on the macroscopic and microscopic scale. An electromagnetic (EM) navigation sensor and OCT optics were incorporated into a single catheter. The EM sensor and baseline CT provide spatial guidance to the SPN and OCT enables volumetric microscopic assessment. The pre-procedure high resolution CT scan was processed to generate the virtual environment. Navigation of the EM-OCT catheter position to the SPNs was tracked using the EM sensor, and OCT images were obtained for final confirmation and tissue assessment. Software was built to reconstruct the true 3D microscopic environment, and to provide real-time guidance of the EM-OCT catheter within the tracheobronchial tree. Validation studies were performed using a lung phantoms and a fixed swine lung.

8927-42, Session 10

Structural and polarization sensitive optical frequency domain imaging with motorized endoscopic catheter

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We present here a high speed three dimensional endoscopic Optical Frequency Domain Imaging (OFDI) system with a miniature motorized catheter. The catheter has a 1.65 mm outer diameter, a 3,000 rpm rotation speed, a focal Full Width at Half Maximum of 9.6 μm and a working distance of 0.47 mm. It is integrated in a high speed polarization sensitive OFDI setup at 1310 nm. We demonstrated its performance with preliminary in vivo structural images of goat bronchial and ex vivo phase retardance images of chicken muscle and tendon. We also quantified the effect of polarization mode dispersion (PMD) in our system. Future work includes testing the polarization sensitive function with in vivo animal bronchial imaging experiment and understanding its significance for lung cancer diagnosis.

8927-43, Session 11

Depth-resolved imaging of diffusing gold nanorods in airway mucus with polarization-sensitive OCT

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Pulmonary mucus plays a vital role in protecting respiratory airway epithelium by trapping inhaled pathogens, toxins and particulates. Mucociliary clearance involves the synchronous and periodic beating of cilia lining the airway epithelium. OCT can quantify the mucus flow rate and monitor relative changes in the ciliary beat frequency in in vitro human broncho-epithelial air-liquid interface (ALI) cultures. However, normal mucociliary clearance is disrupted in diseases such as cystic fibrosis and chronic obstructive pulmonary disease, and mucus associated with these diseases is characterized by higher mucin concentration and lower solvent concentration. This changes mucus viscoelastic properties and makes clearance of mucus from airways difficult. However, methods to characterize probe diffusion and mucus viscoelasticity that can easily be translated to in vivo conditions are lacking. Here we discuss the implementation of polarization-sensitive OCT (PS-OCT) using PEG-coated gold nanorods (GNRs) as diffusion probes to sense changes in mucus concentration ranging from physiologically normal to diseased. We image diffusing GNRs in mucus over a transporting ALI culture, and the exclusion of GNRs from the peri-ciliary layer (PCL). Depth-resolving the co- and cross- polarized autocorrelations of the intensity fluctuations is shown to discriminate rapidly diffusing GNRs and slower motile activities in the PCL. The diffusion of GNRs in mucus measured over the transporting ALI culture was observed to be in agreement with measurements in stationary mucus, which shows the measured diffusion rate of GNRs to be unchanged by the predominantly transverse transport of the mucus.

8927-44, Session 11

Imaging the cellular dynamics in the airways by optical coherence microscopy (OCM)

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Intravital imaging provides insight in tissue architecture und dynamic behavior of tissue components with subcellular resolution. Confocal microscopy has already successfully entered the clinical setting. However the imaging depth is limited by the penetration of short wavelength excitation. Multiphoton microscopy combines ultimate microscopic resolution with high imaging depth and low photodamage, but in inherently slow.

Hence, we investigated the use of high-NA optical coherence microscopy (OCM), which uses high-numeric imaging with low-coherence interferometry, as a new modality for imaging air-ways in

mice. A Thorlabs Hyperion OCT device was adapted to self-build 0.8 NA microscope. Explanted mice tracheas in culture medium as well as anesthetized and ventilated mice with exposed trachea were imaged by OCM. For comparison similar tissue were imaged with autofluorescence based 2-photon microscopy (AF-2PM).

OCM was able to visualize the whole thickness or the trachea wall with approximately 1 µm lateral und 2 µm axial resolution. Cellular structures (e.g. epithelial cells, immune cells, and chondrocytes) and fibrous structure of the connective tissue were successfully imaged. Lymph and blood vessels with individual blood cells were also visible. Image quality of connective tissue was comparable to AF-2PM. However, images of the epithelial cells suffered from significantly lower contrast compared to the 2PM images. In contrast, Cilia of the epithelial cells were seen with good contrast and we were able quantify their beating frequency.

High-NA OCM has great potential for studying cilia function and possibly also immunological function of the airways in vivo. It may also improve clinical diagnosis.

8927-45, Session 11

Dual-modality ?OCT and fluorescence imaging of the CF airway

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Cystic fibrosis (CF) airway disease is characterized by impaired mucociliary clearance, chronic bacterial infection and inflammation in the lung. The genetic cause of CF disease is linked to mutations of the cystic fibrosis transmembrane conductance regulator (CFTR), but the mechanism of progression from defective ion transport to advanced lung disease is not well understood. Several microanatomical and mechanical features are affected in CF, including depleted airway surface liquid (ASL) and periciliary layer (PCL) depths, reduced mucociliary transport (MCT) velocity, and slow ciliary beat frequency (CBF). Salt homeostasis and associated mucus hydration is of key importance as CFTR is a known chloride channel. CFTR also regulates airway pH and bicarbonate concentration which modulate bacterial killing and mucogenesis, respectively. Causative relationships between these physical and chemical parameters during disease progression are unclear due to lack of quantitative tools suitable for in situ studies.

Here we present a dual-modality micro-optical coherence tomography (?OCT) and confocal fluorescence microscope for simultaneous study of depth-resolved airway microanatomy, mechanical properties of ciliary motion and mucus transport, and local chemistry in situ. Relevant chemical parameters are obtained with functional fluorescent dyes such as BCECF, which has been successfully used to study intracellular pH in vivo, and through the Henderson-Hasselbalch relation, which yields bicarbonate concentration. The combined system allows longitudinal study of CF and non-CF cell and tissue culture, and will be a useful tool for elucidating causative relationships between the above listed parameters during the pathogenesis of CF, as well as monitoring effects of pharmacological intervention.

8927-46, Session 11

OCT-based quantification of ciliary flow defects generated by gene knockdown

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The development of normal ciliated respiratory epithelium is important for maintaining pulmonary health. One important but poorly understood aspect of ciliary physiology is how disruption of molecular biological pathways, e.g. altered gene expression, can translate into intermediate, quantitative defects in the ability of respiratory epithelium to clear mucus. The ciliated epithelium of Xenopus embryos is a particularly important model system for addressing the downstream physiological consequences of gene expression because it provides ready access to a natural ciliated surface while allowing for relatively facile genetic manipulation. We previously showed that it was possible to quantify ciliary performance in Xenopus embryos using optical coherence tomography (OCT)-based particle tracking velocimetry. Here, we show results of manipulation of three genes in Xenopus. First, using antisense morpholino technology, we knocked down both dynein axonemal heavy chain (dnah9), a motor protein involved in ciliary movement, and kinesin family member 3A (kif3a), a protein involved in ciliary synthesis. Knockdown of dnah9 resulted in intermediate flow defects, with ciliary flow monotonically decreasing with the dosing of the morpholino. Preliminary data on kif3a knockdown yielded a relatively constant flow over most dosing until abruptly dropping at a certain threshold, consistent with a binary phenotype. Additionally, we investigated the use of speckle variance to identify ciliary patches in animals overexpressing the notch intracellular domain, a gene that plays an important role in patterning and density of ciliation. Lastly, building on this work in Xenopus, we are continuing to investigate ciliary flow in the neonatal mouse trachea using OCT.

8927-47, Session 11

Micro-optical coherence tomography study of murine trachea explant model of regeneration after injury

Kengyeh K. Chu, Vladimir Vinarsky, Adam Lam, Massachusetts General Hospital (United States); Andy Wu, Univ. of Tokyo (Japan); Jayaraj Rajagopal, Guillermo J. Tearney, Massachusetts General Hospital (United States)

Healthy mammalian airways are lined with cilia, microscopic organelles of the epithelium approximately 7 ?m in length and typically in rapid motion, beating several times per second to clear debris-containing mucus out of the airway. Upon certain forms of physical injury, the ciliated epithelium in an airway can be lost, but will regrow and regain its cilia and re-establish mucociliary transport in a matter of days to weeks. Recent development of culturing technique has enabled the growth of mouse trachea explants ex vivo for periods exceeding 7 weeks, making these tissue cultures a promising model for longitudinal study of injury and regeneration. Tracheas are injured in vivo with sulfur dioxide such that the ciliated epithelium is removed, then explanted and allowed to regenerate in vitro.

To study ciliary regeneration in injured murine trachea explants, we conducted a longitudinal imaging study using high resolution micro-optical coherence tomography (?OCT). ?OCT has been previously demonstrated for functional imaging of animal airway epithelia and mucociliary transport. ?OCT yields 2 ?m lateral and 1 ?m axial resolution that enable direct visualization of the cilia. Quantitative measurements of airway function, including ciliary beat frequency (CBF) and mucociliary transport (MCT) rate, can be extracted from image sequences. Epithelial thickness was also tracked over time.

We present longitudinal once-daily imaging results of 8 injured trachea explants over 2 weeks. Mucociliary clearance was recovered in each specimen by day 14; moreover, in each case, the original direction of transport was preserved, despite the complete removal of ciliated epithelium upon injury, suggesting an underlying mechanism for ciliary axis memory independent of ciliated cell loss. Comparisons with in vivo recovery specimens demonstrate a similar trajectory of regeneration, validating the ex vivo trachea explant model for regeneration. The combination of durable tissue culture technique and ?OCT imaging will

enable detailed long-term studies that provide new insights into the mechanisms governing cilia regeneration.

8927-48, Session 12

Photodynamic inactivation of microorganisms which cause pulmonary diseases with infrared light: an in vitro study

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Lower respiratory infections are among the leading causes of mortality worldwide, being responsible for 1.5 million deaths every year (WHO, 2006). One of the problems associated with these diseases, e.g. pneumonia, is the growing microbial resistance to antibiotics, which reduces the conventional treatment effectiveness and creates a necessity of developing new approaches. Photodynamic therapy is based on oxidation of cellular components by reactive oxygen species produced when photosensitizers are activated by light in an oxygen-rich environment. In this study, it was evaluated the interaction of indocyanine green (ICG), a photosensitizer activated by infrared light, with alveolar macrophages (AM). Initial experiments analyzed ICG toxicity to AM in the dark with different drug concentrations (9.37, 18.75, 37.5, 75, 150 and 300 μM) and incubation times (10, 20 and 30 minutes). AM viability was obtained indirectly via the MTT method, and results revealed that, for 10 and 20 minutes, AM viability remains around 93% (excepting 150 μM for 10 minutes and 300 μM for 20 minutes, which yielded 89.1 and 82.4%, respectively). For 30 minutes, viability values oscillate around 93%. However, 150 and 300 μM have yielded values of approximately 76.5% and, due to this low rate of viability, further experimental procedures will be conducted excluding the highest concentration of ICG in order to minimize AM damage. Also, further experiments with Streptococcus pneumoniae alone and in culture with AM will be conducted verifying the photodynamic inactivation effectiveness of the tested drug concentrations and incubation periods using infrared light.

8927-49, Session 12

Pulmonary decontamination for photodynamic inactivation with extracorporeal illumination

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Infectious pneumonia is a major cause of morbidity and mortality, despite advances in diagnostics and therapeutics in pulmonary infections. One of the major difficulties associated with this infection is due the high rate of antibiotic resistant microorganisms. The photodynamic inactivation is emerging as one of the promising possibilities in this area, because its action is oxidative, not allowing microorganism to develop resistance to treatment. Photodynamic inactivation for pulmonary decontamination has potential for disease treatment or to create better conditions for antibiotics action. In this study, we are developing a technique for lung diseases treatment by photodynamic inactivation with extracorporeal illumination. Firstly measurements using phantom model were performed to simulate light penetration in biological tissues at various fluency rates. The temperature and the transmittance of light in an ex vivo model were analyzed. It was used an 810 nm laser beam in continuous mode. Our previous results showed a 50% of leakage at 0.5 mm of thickness in phantom model. The temperature variation was 5.4 °C in ex vivo model and was observed a significant transmittance of the light occurs in mice chest. These results are suggesting the possible application of extracorporeal illumination with infrared light source. Further studies will be performed in animal model using indocyanine green and bacteriochlorin as sensitizers. The pulmonary infection will be induced by Streptococcus pneumoniae and Klebsiella pneumoniae.

8927-50, Session 12

Automated segmentation and quantification of lung structures from optical coherence tomography images

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Many lung diseases, such as emphysema and pulmonary fibrosis, produce characteristic changes in the structures of the lung, leading to the impairment of lung function. Optical coherence tomography (OCT) has been shown capable of visualizing these lung structures, such as alveoli. However, characterising significant areas of lung tissue through manual measurements is infeasible due to the extremely large number of airway structures present.

We present a fully automated segmentation and quantification algorithm capable of delineating large numbers of lung structures, including individual alveoli and acinar air spaces. Segmentation is based on the level set method, and includes a number of pre- and post-processing steps optimized for OCT images. Having identified the lung structures, we automatically characterize the lung tissue using an adapted stereological technique to calculate the median chord length across each segmented air space. This provides a method to quantify the geometry of the lung tissue. This algorithm is demonstrated using scans acquired with OCT needle probes in fresh, ex vivo tissue from two healthy animal models: pig and rat; and 10,000 automated measurements were computed on each scan. The automated estimates of median airspace size were within 5 microns of manual measurements. The algorithm also enabled calculation of 3D volume renderings to visualize the segmented lung structures.

8927-51, Session 12

Semi-automated segmentation of porcine airway wall layers using optical coherence tomography: comparison with manual segmentation and histology

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Rationale: Optical coherence tomography (OCT) is a novel, micron-scale resolution technique for airway imaging, but it relies on manual tracing of the airway to obtain measurements. The objective of this study was to develop semi-automated OCT airway wall layer measurements and compare results with manual segmentation and histology.

Methods: OCT imaging of excised porcine airway specimens was performed using a 1.5mm probe. Specimens were subsequently prepared

for histology and manual measurements of airway lumen (Ai), outer wall area (Ao) and wall area (Aaw) were performed for both histology and OCT using ImageJ (NIH, USA). Semi-automated OCT image segmentation was performed in ImageJ; pre-processing was performed using a median filter with radius=100 and contrast limited adaptive histogram equalization. A median filter with radius=4 and a bandpass filter were then applied to reduce image noise and enhance edges. Segmentation of the Ai as well as the cartilage and lamina propria layers were automatically performed by level set method segmentation with seed point initialization. Aaw was segmented by applying a morphological closing operation on the union of all segmentation masks.

Results: For the 6 images evaluated, there was no significant difference between Aaw generated using OCT semi-automated and manual (automated=10.7±1.2mm2, manual=11.3±0.40mm2, p=0.16) or between OCT semi-automated and histology measurements (histology=9.71±0.38mm2, p=0.16). For OCT semi-automated segmentation, the cartilage and lamina propria area were 2.15±0.43mm2 and 2.12±0.28mm2, respectively.

Conclusions: Semi-automated segmentation of airway wall layers using OCT is feasible and shows good quantitative agreement with manual segmentation and histology.

8927-52, Session 12

Micro-optical coherence tomography assay of leukocyte migration through epithelial monolayers

Kengyeh K. Chu, Mark E. Kusek, Eric Wilsterman, Bryan P. Hurley, Guillermo J. Tearney, Massachusetts General Hospital (United States)

High resolution micro-optical coherence tomography (?OCT) has been shown to be a versatile tool in a variety of organ systems. The high resolution of ?OCT allows direct visualization of individual cells and micron-scale features of the cellular environment without exogenous contrast agents. Previously, ?OCT has been used to study in vitro models of respiratory epithelium as a model for airway disease, and a high-throughput ?OCT drug screening system for human bronchial epithelial (HBE) cells was reported in which quantitative metrics of epithelial function were derived from ?OCT images.

We now demonstrate a ?OCT imaging method for quantifying leukocyte migration in an immune response assay. A suspension of immune blood cells is added to the basal compartment of an epithelial culture, and ?OCT volume scans of the monolayer and the apical compartment are acquired over time. Individual cell migrations are observed, and statistical observations derived from these images are useful for quantitatively evaluating modulators of immune response and inflammation.

The ?OCT migration assay has been demonstrated with H292 respiratory epithelial monolayers on Corning Transwell filters. Migration of neutrophils was measured in response to the chemoattractant fMLP and negative control. Several regions of 0.25 mm-squared on each monolayer were scanned for two hours at 10 minute intervals. Neutrophil migration into the apical space is determined at each time point by measuring the number of voxels over a threshold intensity in the apical compartment, normalized by the number of above-threshold voxels of isolated neutrophils. Peak fMLP-driven migration occurred at 40 minutes (1417 cells ± 85 SEM, n=4) compared to control at the same time point (140 cells ± 17 SEM, n=4, p<0.00001).

Because migration kinetics are captured non-destructively and only minute quantities of leukocytes and cell surface area are required, ?OCT migration imaging is a powerful immune response assay even when the available quantity of the reactants is limited, and will provide new insights into the process of immune response and inflammation.

8927-54, Session 13

Towards the diagnosis of primary lung carcinomas with optical coherence tomography

Lida P. Hariri, Mari Mino-Kenudson, Michael Lanuti, Alyssa J. Miller, Matthew B. Applegate, Melissa J. Suter, Massachusetts General Hospital (United States)

Recent developments in targeted lung cancer therapies have increased the demand for tumor tissue to obtain both histological and molecular diagnostics. However, transbronchial biopsy and needle aspiration often have variable tumor yields. Optical coherence tomography (OCT) has the potential to guide biopsy site selection and improve diagnostic yield. We have previously shown that OCT can distinguish lung nodules from parenchyma with sensitivity and specificity >95%. We now aim to assess whether OCT can provide additional “virtual” tissue to aid in diagnosing lung carcinomas. In this study, we develop and validate OCT criteria for squamous cell carcinoma (SCC), adenocarcinoma, and poorly differentiated carcinoma. A total of 78 tumor samples (36 adenocarcinoma, 23 SCC, 19 poorly differentiated carcinoma) from 37 ex vivo resection specimens were included in a blinded assessment with 3 independent readers (one pathologist, pulmonologist, and OFDI expert). OCT of SCC requires the presence of rounded or irregularly shaped, signal-intense nests. Adenocarcinoma requires both the presence of small, round signal-poor structures and a lack of signal-intense nests. Poorly differentiated carcinoma requires a lack of both signal-intense nests and small, round signal-poor structures. The overall accuracy was 81.2% (range: 80.8-82.1%). The accuracies for adenocarcinoma, SCC, and poorly differentiated carcinoma were 80.6% (range: 72.2-88.9%), 72.4% (range: 65.2-87.0%), and 93.0% (range: 89.5-100%), respectively. The wide range in accuracies suggests that there is potential for improvement with further training. These results indicate OCT has potential to aid in diagnosing lung carcinomas, but as a complement to, rather than a replacement of, traditional tissue biopsy.

8927-55, Session 13

Distinguishing tumor from tumor-associated fibrosis in pulmonary nodules with polarization-sensitive optical coherence tomography

Lida P. Hariri, David C. Adams, Martin L. Villiger, Brett E. Bouma, Alyssa J. Miller, Matthew B. Applegate, Mari Mino-Kenudson, Melissa J. Suter, Massachusetts General Hospital (United States)

In light of emerging targeted lung cancer therapies, bronchial biopsy and transbronchial fine needle aspiration (TBNA) must obtain sufficient tumor volumes for both histological and molecular diagnostics. However, these techniques can yield insufficient tumor volumes, in part due to inadvertent biopsy of tumor-associated fibrosis. The ability to assess biopsy sites with optical coherence tomography (OCT) during bronchoscopy is likely to improve diagnostic yield. We have previously demonstrated that OCT can distinguish lung nodules from parenchyma with high sensitivity and specificity (> 95%), but structural OCT cannot differentiate solid tumor from fibrosis within nodules. Polarization sensitive OCT (PS-OCT) measures birefringence of organized tissues, such as collagen, and could dramatically increase diagnostic yield by distinguishing tumor from fibrosis. In this study, PS-OCT was obtained in 97 lung nodule samples from 32 ex vivo specimens containing varying amounts of tumor and fibrosis. PS-OCT was obtained with either a custom-built 2.4 Fr (0.8mm diameter) helical scanning catheter or a dual-axis bench top scanner. Strong birefringence was present in regions with dense, established fibrosis, with no birefringence in regions of tumor. Tumors with admixed early, loosely-organized collagen showed mild to moderate birefringence, and tumors with little connective tissues showed

little to no birefringent signal. PS-OCT provides further insights into lung nodule composition than is currently appreciable with standard structural OCT. The ability to differentiate tumor from tumor-associated fibrosis supports the potential of PS-OCT to guide biopsy site selection during bronchoscopy and increase diagnostic tumor yield.

8927-56, Session 13

Imaging airway smooth muscle with PS-OFDI

David C. Adams, Lida P. Hariri, Alyssa J. Miller, Martin L. Villiger, Brett E. Bouma, Melissa J. Suter, Massachusetts General Hospital (United States)

Present understanding of the pathophysiological mechanisms of asthma has been severely limited by the lack of an imaging modality capable of assessing airway conditions of asthma patients in vivo. Of particular interest is the role that airway smooth muscle (ASM) plays in the development of asthma and asthma related symptoms. Here we present the first ever imaging of ASM using Polarization Sensitive Optical Frequency Domain Imaging (PS-OFDI), and conclusively demonstrate that this technology offers enormous potential as a minimally invasive means of assisting in the study of asthma.

OFDI is a second-generation variation of Optical Coherence Tomography (OCT) which benefits from both fast imaging speeds and high sensitivity. With standard OFDI, however, identifying ASM is often not possible due to poor structural contrast. By incorporating polarization-sensitive detection into the system, we are able to substantially increase the contrast of a segment of ASM, which naturally exhibits a high degree of birefringence relative to the surrounding tissue. Using both bench-top and catheter-based approaches, we demonstrate the ability of PS-OFDI to image ASM in segments of porcine airways, over a range of airway sizes. Calculation of ASM burdens and precise correlation with histology provide significant evidence that PS-OFDI offers accurate ASM imaging potential. Additionally, we present in vivo data taken in the airways of human subjects, thereby demonstrating the potential applicability of this technology to human patients.

8927-57, Session 13

Validation of micro-optical coherence tomography particle tracking rheology

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In cystic fibrosis (CF) lung disease, current understanding of the pathogenic progression from CF transmembrane conductance regulator (CFTR) defect to advanced lung disease contains missing links despite considerable research. Many intermediate defects are known to occur, including delayed mucociliary transport, decreased defense against infection, and elevated mucus viscosity. Establishment of causal links between these observations has remained elusive, in large part due to the lack of technology capable of interrogating airways in vivo at a microscopic scale. Elevated viscosity in particular is suspected to debilitate mucociliary transport and invite infection in a commonly invoked hypothesis, but evidence has been limited due to inability to measure mucus viscosity in situ. Furthermore, the study of mucus viscosity is hampered by conventional rheometric techniques that require more mucus than can readily be extracted from either in vitro model cultures or in vivo.

We have previously demonstrated that high resolution micro-optical coherence tomography (?OCT) can be used to track particulate inclusions in expectorated sputum, providing a measure of viscosity by measuring the resistance of the mucus to diffusion of the particles. We found that diffusion in expectorated sputum from CF donors was of significantly lower magnitude than in normal sputum, indicating elevated viscosity.

We now present a method for the computation of dynamic viscosity as a function of frequency using ?OCT particle tracks, which is directly comparable to the output of conventional cone-and-plate rheometers. The ?OCT rheology algorithm implements bulk motion subtraction to eliminate spurious contributions from non-diffusive motion, and also suppresses the effect of particle localization error that would otherwise dominate the small amplitude diffusion of particles in high-viscosity media. We validate our ?OCT rheological method by measuring known viscosity standards and comparing with fluorescent particle-tracking rheometry. Accurate rheology using ?OCT will enable the study of the mechanistic relationship between mucus viscosity and airway function using images from a single modality.

8927-58, Session 13

Optical monitoring of respiratory muscles hemodynamics and oxygenation in response to resistive breading

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Oxygenation and hemodynamics responses of the primary and secondary inspiratory muscles during loaded breathing have not been extensively studied.

Determining the amount of loading that results in significant changes of muscle oxygenation of the inspiratory muscles provide important insight into their respective contribution to loaded ventilation in healthy people as well as provide a foundation for investigating parallel issues in chronic respiratory disease (COPD).

Using a four-channel continuous-wave near-infrared spectroscopy (NIRS) we investigated the pattern of changes in muscle oxygenation, deoxygenation and blood flow in the sternocleidomastoid (SCM), the parasternal (PS) and intercostal (IC) muscles during a bout of incremental inspiratory threshold loading (ITL) in healthy subjects, by measuring light attenuation at 760 and 864 nm wavelengths that was analyzed using algorithms based on the modified Beer–Lambert law.

During progressive loading, the PS and IC showed a significant increase in oxygenated hemoglobin and the SCM showed an increase in deoxygenated hemoglobin. NIRS-derived local blood volume, also steadily increased in the SCM whereas it decreased in the quiescent control muscle during the ITL. Our data suggests that the SCM is recruited progressively during progressive ITL and is accompanied by an increased blood volume and maintenance of oxygenated hemoglobin.

NIRS has potential to advance our understanding of human respiratory muscle oxygenation and hemodynamics during increased ventilatory demands in healthy individuals and those with respiratory disease. The clinical importance of monitoring the SCM in COPD patients may be most obvious in those who undergo weaning from mechanical ventilation after suffering from respiratory failure.

Conference 8928A: Optical Techniques in Neurosurgery, Brain Imaging, and Neurobiology

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8928 Optical Techniques in Neurosurgery, Neurophotonics, and Optogenetics

8928-1, Session 1

Microendoscopy of the epileptic brain using combined confocal fluorescence microscopy and Doppler optical coherence tomography

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Epilepsy affects about 50 million people worldwide; 30% of which are unresponsive to treatment. With the increasing use of hemodynamic imaging techniques to characterize the epileptic brain, a clear link between the hyperactivity of neurons and ensuing hemodynamic signals is required to better understand these signals.The goal of this work is to develop a microscopic imaging system measuring both neuronal activity and hemodynamics and then investigate its performance in vivo in an animal model of epilepsy.[[ ]]

We designed a multimodal endoscopeallowing micron level resolution(confocal fluorescence microscope or CFM) and high penetration depth(Doppler optical coherence tomography or DOCT). A common imaging arm for both modalities includesa 2D galvanometer system for 2D imaging in CFM and 3D imaging in DOCT, and is terminated by a GRIN triplet. The triplet was designed to relay light deeply into the brain and maximize resolution. An alternative design with a prism and a mirror at the end of the triplet for side view imaging is also presented. The common imaging arm can be installed on a stereotaxic holder or freely handled during the imaging session, as light collection is performed through optical fibers.[[ ]]

We imaged neuronal activity with CFM at 473 nm using a Ca2+ indicator (Oregon Green Bapta-1) while simultaneously monitoring brain hemodynamics with DOCT at 870 nm. Imaging of the epileptic mouse brain was achieved in vivo by inserting the GRIN endoscope through a craniotomy window.[[ ]]

8928-2, Session 1

Transparent cranial implant for non-invasive, chronic access to brain for optical diagnostics and therapeutics

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Transparent nanocrystalline Yttria-Stabilized-Zirconia (nc-YSZ) represents a novel cranioplasty material that provides biomechanical stability for brain protection as well as non-invasive and chronic optical access to the brain for delivering and/or collecting light into the deep brain for neuroscientific and neuroengineering applications such as optogenetics, photodynamic therapy, and low-level-laser therapy.
To evaluate the potential of nc-YSZ implant for optical therapy and imaging, three parallel studies were performed. First, we investigated the inflammatory and angiogenic host tissue response to nc-YSZ for two weeks using a hamster dorsal skinfold chamber model. Intravital flouorescence microscopy of host tissue revealed no significant change in angiogenesis, microhemodynamics, microvascular permeability, and leukocyte-endothelial cell interaction. Second, we assessed the optical functionality of the nc-YSZ using optical coherence tomography (OCT) to compare the signal intensity of murine brain through native skull and the transparent nc-YSZ. OCT imaging of murine brain, in vivo, indicated an increase in signal strength when imaging through nc-YSZ compared to native cranium. Third, we determined the thermal functionality of nc-YSZ using a brain phantom with embedded thermocouples. Measurement of fluence rate and temperature distributions in the phantom suggested that while non-linear photon propagation and energy disposition in nc-YSZ differs from native cranium, the maximum temperature increase stayed below the threshold for heat-induced coagulation. Therefore, routine imaging and therapy procedures can be performed without inducing thermal damage to the brain covered with nc-YSZ. Thus, using nc-YSZ as cranioplasty material allows for non-invasive, chronic delivering and/or collecting light to/from the brain for optical diagnostic and therapeutic applications.

8928-3, Session 1

Optical coherence microscopy of mouse cortical vasculature surrounding implanted electrodes

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Optical coherence microscopy (OCM) provides real-time, in-vivo, three-dimensional, isotropic micron-resolution structural and functional characterization of tissue, cells, and other biological media or targets. OCM also provides visualization and quantification of vascular flow via phase-resolved techniques. Several groups have used speckle variance and phase-sensitive techniques, among others, to image mouse cortical tissue for neurological applications. The Center for Devices and Radiological Health at the Food and Drug Administration has a research program with the objective to examine the underlying mechanisms of chronic functional degradation of neural prostheses. Angiogenesis, capillary network remodeling, and changes in flow velocity are potential indicators of tissue changes that may be associated with waning electrode performance. The aim of the current investigation is to quantify longitudinal changes in vascular morphology and capillary flow around neural electrodes chronically implanted in mice. We built a 1300-nm OCM system to image vessels in neocortical tissue in a cohort of mice. The mouse motor cortex was imaged through an optical window implanted on the skull. A single electrode was inserted into the cortex at an oblique angle to the window. The mice were imaged with OCM weekly and vascular maps in the region around the electrode were generated. Calculation of adjacent B-scan phase difference provided optimal visualization of capillaries because the time interval matched their lower flow velocities, though bulk motion artifacts were problematic. Preliminary results from several mice will be presented, as well as solutions to overcome phase noise and other instrumentation and preparation challenges.

8928-4, Session 1

Cerebral metabolism in the rodent cortex measured with 2-photon fluorescence-lifetime microscopy of NADH

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Understanding brain metabolism at a cellular level is important to characterize brain function under healthy and diseased conditions. Here we present minimally-invasive, in-vivo measurements of reduced nicotinamide adenine dinucleotide (NADH) fluorescence as an indicator of cerebral metabolism, collected in vivo from the exposed cortices of rats and mice. Commercially available time-correlated single photon counting (TCSPC) equipment was integrated into our custom-built multimodal imaging system, enabling 2-photon fluorescence lifetime imaging (FLIM) at designated points in cerebral tissue with high spatial and temporal resolution. Measurements were performed in both neurons and astrocytes during baseline conditions and during transient physiological perturbations such as hypoxia, anoxia, and injection of metabolic inhibitors. Multi-exponential fits for NADH fluorescence lifetimes indicate multiple, distinct enzyme-bound formulations, or 'species.' Consistent with our observations of cerebral oxygen tension in brain tissue, NADH fluorescence varies with proximity to vasculature. Each NADH specie responds differently to hypoxia and anoxia. Compared to traditional intensity-based NADH measurements, lifetime imaging of NADH is less susceptible to the adverse effects of overlying blood vessels. NADH FLIM could yield more comprehensive information than NADH intensity, enabling better distinction of anaerobic from aerobic activity. Evaluating NADH measurements will ultimately lead to a deeper understanding of cerebral energetics and its pathology-related alterations. Such knowledge will aid development of therapeutic strategies for neurodegenerative diseases such as Alzheimer's Disease, Parkinson's disease, and stroke.

8928-5, Session 2

Interpreting CARS images of tissue within the C-H-stretching region

Benjamin Dietzek, Tobias Meyer, Anna Medyukhina, Norbert Bergner, Christoph Krafft, Institut für Photonische Technologien e.V. (Germany); Michael Schmitt, Friedrich-Schiller-Univ. Jena (Germany); Jürgen Popp, Institut für Photonische Technologien e.V. (Germany)

Single band coherent anti-Stokes Raman scattering (CARS) microscopy is the fastest implementation of vibrational imaging of tissue. This approach allows for video-rate image acquisition, if the strong Raman resonances in the CH-stretching region are employed. However, this choice of Raman resonance is generally considered to yield only limited chemical specificity due to the ubiquitous appearance of CH2/CH3 groups in cells and tissue. Here we present an example in which CARS imaging within the CH-stretching region is applied to detect individual cells and nuclei of human brain tissue and brain tumors – an information which allows for histopathologic grading of the tissue.

In this contribution CARS image contrast within the C-H-stretching region is interpreted by direct comparison with Raman mapping and correlated to the tissue composition justifying the use of CARS imaging in this wavenumber region for biomedical applications. Based on the specific application example of identifying nuclei within (coherent) Raman images of neuro-tissue sections, we shall derive general design parameters for laser systems optimally suited to serve in a clinical environment and discuss the potential of recently developed methods for (i) analysis of spectrally resolved CARS images and (ii) image segmentation algorithms.

This work was financially supported by the European Union via the Europäischer Fonds für Regionale Entwicklung (EFRE) and the "Thüringer Ministerium für Bildung Wissenschaft und Kultur (TMBWK)" (Projects: B714-07037, B578-06001, 14.90 HWP) and via the European network of excellence P4L (Photonics4Life) as well as by the German Ministry for Science and Education (BMBF) MediCARS (FKZ: 13N10774) is highly acknowledged.

8928-6, Session 2

Enhancing contrast of brain neoplasms using dye-enhanced wide-field high-resolution optical imaging

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We have employed a wide-field high-resolution imaging system for the analysis of brain specimens. Demeclocycline, a fluorescing antibiotic, was used to enhance contrast of optical images. Fresh samples were obtained immediately after surgeries, stained in 0.25 mg/mL of DMN and imaged. The samples imaged included both normal and pathological brain tissue. In total, we have imaged 6 pathological specimens, including 3 glioblastomas, pituitary adenoma, choroid plexus carcinoma, and a meningioma. Normal brain specimens included white brain matter and gray brain matter. The specimens were interrogated by monochromatic diode laser light at 402 nm. Reflectance signal at 402 nm and fluorescence signal between 455 nm and 495 nm were collected using a wide-field and high-resolution imager. Wide-field images, with a field of view of 3 cm x 4cm, lateral resolution of 12 ?m, and axial resolution of 50 ?m were first acquired to determine what areas to image with higher resolution. High-resolution confocal images provided axial resolution of 3-5 ?m and lateral resolution of better than 0.9 ?m with a field of view of 250 ?m. After the samples were imaged, H&E histopathology was processed from the areas imaged and correlated with the acquired optical images. The high-resolution reflectance and fluorescence optical images provide complimentary information on tissue morphology and dye uptake, respectively. Our results indicate that wide-field high-resolution imaging approach is promising for guiding surgical procedures of the brain.

8928-7, Session 2

Raman spectroscopy of gliomas: an exploratory study

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Gliomas are extremely infiltrative type of brain cancers, the borders of which are difficult to locate. Gliomas largely consist of tumors of astrocytic or oligodendroglial lineage. Usually stereotactic surgery is performed to obtain tumor tissue sample. Complete excision of these tumors with preservation of uninvolved normal areas is important during brain tumor surgeries. The present study was undertaken to explore feasibility of classifying abnormal and normal glioma tissues with Raman spectroscopy (RS). RS is a nondestructive vibrational spectroscopic technique, which provides information about molecular composition, molecular structures and molecular interactions in tissue. Post-operated 33 (20-abnormal and 13-normal) gliomas tissue samples of different grades were collected under clinical supervision. Five micron section from tissue sample was used for confirmatory histopathological diagnosis while the remaining tissue was placed on CaF2 window and spectra were acquired using a fiberoptic-probe-coupled HE-785 Raman-spectrometer. Spectral acquisition parameters were laser power-80mW, integration-20 s and averaged over 3 accumulations. Spectra were preprocessed and subjected to unsupervised Principal-Component Analysis (PCA) to identify trends of classification. Supervised PC-LDA (Principal-Component-Linear-Discriminant Analysis) was used to develop standard-models using spectra of 12 normal and abnormal specimens each. Leave-one-out cross-validation yielded classification-efficiency of 90% and 80% for normal and abnormal conditions, respectively. Evaluation

with an independent-test data-set comprising of 135 spectra of 9 samples provided sensitivity of 100% and specificity of 70%. Findings of this preliminary study may pave way for objective tumor margin assessment during brain surgery.

8928-8, Session 3

Quantitative, spectrally-resolved intraoperative imaging system for neurosurgical guidance in brain tumor surgery: pre-clinical and clinical results

Pablo A. Valdes, Valerie L. Jacobs, Dartmouth College (United States); Frédéric Leblond, Ecole Polytechnique de Montréal (Canada); Brian C. Wilson, Univ. of Toronto (Canada); Keith D. Paulsen, Dartmouth College (United States); David W. Roberts M.D., Dartmouth Hitchcock Medical Ctr. (United States) and Dartmouth College (United States)

The goal of surgical resection of brain tumors is to maximize the extent of tumor removal, which has been repeatedly demonstrated to correlate with improved patient survival and quality of life. Fluorescence guided surgery has been shown as a useful adjunct in achieving this goal. State-of-the art commercial surgical microscopes modified for fluorescence imaging are currently used with protoporphyrin IX, fluorescein, and indocyanine green as the contrast agent, but demonstrate the following key limitations: subjective assessments (i.e., qualitative assessment of fluorescence without correcting for the distorting effects of tissue optical properties); low sensitivity (i.e., significant levels of fluorescence-positive tumor are undetected); and non-spectrally resolved (i.e., bandpass/ longpass systems without spectrally-resolved detection for simultaneous, multiple fluorophore imaging). Here, we present an intraoperative, quantitative, and spectrally-resolved imaging system that integrates seamlessly onto commercial neurosurgical microscopes. We perform spectrally resolved detection in the range 400-1100 nm, enabling detection of visible and NIR fluorophores, and apply a normalization algorithm to partially correct for the distorting effects of tissue optical properties, enabling quantitative assessment of tissue fluorescence. In pre-clinical studies in rodent intracranial models of glioma, we present imaging of multiple fluorophores, including PpIX, fluorescein and NIR activatable probes, as well as clinical results in patients undergoing brain tumor resection, demonstrating improved tumor detection. This translational approach enables spectrally-resolved fluorescence imaging that easily integrates into the neurosurgical workflow for brain tumor surgery and opens the door to simultaneous imaging of multiple fluorophores in the visible and NIR range.

8928-9, Session 3

Through-microscope spectroscopic excitation and emission for fluorescence molecular imaging as a tool to guide neurosurgical interventions

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In intraoperative microscopy, quantitative fluorescence imaging has proven to be a powerful tool to guide neurosurgical interventions. However, commercial instruments lack detection sensitivity and are designed to image a single fluorescent tracer, usually indocyanine green, protoporphyrin IX or fluorescein. We introduce here a versatile and modular high-sensitivity spectroscopic imaging system that can be seamlessly connected to commercial neurosurgical microscopes and provide surgeons with spectroscopic fluorescence and reflectance images of the surgical field. The system provides an unprecedented spatial and spectral information content by allowing through-microscope, wide-field images to be acquired hyperspectrally at both excitation and fluorescence emission wavelengths. The instrument is capable of exciting and detecting a large number of visible and near-infrared markers, while measuring tissue autofluorescence throughout the optical range. Illumination is achieved with a supercontinuum laser (?400nm to >1000nm) coupled to a laser-line tunable filter with 3 nm spectral resolution. On the detection side, an imaging bundle directs the fluorescent light to a beam splitter, which channels signals through two liquid-crystal tunable filters with sensitivity either in the visible or the NIR, allowing imaging to be achieved between 400nm and 1100nm. Detection of visible light is done with a scientific grade charge-coupled device (CCD) camera, while NIR detection is done with a highly sensitive electron-multiplying CCD camera. Detailed system characterization studies are presented using liquid phantoms mimicking brain tissue to determine the system sensitivity and specificity, its ability to quantify and simultaneously image multiple fluorescent markers, and its capacity the detect near-infrared fluorophores at depth in tissue.

8928-10, Session 3

Photochemical internalization (PCI) enhanced nonviral transfection of tumor suppressor and pro-drug activating genes; a potential treatment modality for gliomas

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Introduction Despite advances in surgery, chemotherapy and radiotherapy, the outcomes of patients with GBM have not significantly improved. Tumor recurrence in the resection margins occurs in more than 80% of cases indicating aggressive treatment modalities, such as gene therapy are warranted. We have examined photochemical internalization (PCI) as a method for the non-viral transfection of the cytosine deaminase (CD) suicide gene into gliomas cells. The (CD) gene encodes an enzyme that can convert the nontoxic antifungal agent, 5-fluorocytosine (5-FC), into the chemotherapeutic drug, 5-fluorouracil (5-FU) Additionally, 5-FC crosses the blood-brain barrier (BBB).

Methods & Results. Multicell tumor spheroids derived from established rat and human glioma cell lines were used as in vitro tumor models. Plasmids containing either the CD gene alone or together with the uricil phosphoribosyl transferase (UPRT) gene combined with the gene carrier protamine sulfate were employed in all experiments. PCI was performed with the photosensitizer AlPcS2a and 670nm laser irradiance. Protamine sulfate/CD DNA polyplexes proved nontoxic but inefficient transfection agents due to endosomal entrapment. In contrast, PCI mediated CD gene transfection resulted in a significant inhibition of spheroid growth in the presence of, but not in the absences of, 5-FC. Repetitive PCI induced transfection was more efficient at low CD plasmid concentration than single treatment.

Conclusion; The results clearly indicate that AlPcS2a-mediated PCI can be used to enhance transfection of a tumor suicide gene such as CD, in malignant glioma cells and cell transfected with both the CD and UPRT genes had a pronounced bystander effect.

8928-11, Session 3

PDT-induced blood-brain barrier disruption facilitates nanoparticle-loaded macrophage migration into the brain

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Photodynamic therapy (PDT) – induced disruption of the blood-brain barrier (BBB) has been investigated as a technique for the delivery of therapeutic agents to selective regions of the brain. The effects of PDT on the migration of systemically administered exogenous macrophages (Ma) loaded with iron oxide nanoparticles in non-tumor bearing rats was investigated.

A control group consisting of three Sprague-Dawley rats was injected with iron oxide-loaded rat alveolar Ma while two animals were subjected to intracranial injection of iron oxide-loaded Ma. PDT-treated animals were injected with photosensitizer (AlPcS2a; 1 mg/kg i.p.) followed by light irradiation (wavelength = 670 nm; light dose = 2.5 J) 48 h later. Prior to light irradiation, iron oxide-loaded Ma were administered to each animal. Animals in all groups were imaged in a 7 Tesla (T) magnetic resonance (MR) imager to determine the extent of PDT-induced edema and to evaluate for the presence of iron oxide nanoparticles. Animals were sacrificed 7 days post Ma administration and their brains analyzed for the presence of iron oxide using Perls staining.

Significant uptake of iron oxide nanoparticles by rat alveolar macrophages was observed thus providing the rationale for their use as delivery vectors. Histopathological analyses failed to find evidence of iron oxide in normal rat brain suggesting that exogenous Ma are incapable of traversing the normal BBB. Accumulations of iron oxide-loaded Ma were observed in both MR images and histological sections of non-tumor bearing rat brain following PDT-induced disruption of the BBB.

8928-12, Session 3

Mid-IR laser system for neurosurgery

Marc Klosner, Chunbai Wu, Donald Heller, Light Age, Inc. (United States)

We present work on a laser system operating in the near- and mid-IR spectral regions, having output characteristics designed to be optimal for cutting various tissue types. We provide a brief overview of laser-tissue interactions and the importance of controlling certain properties of the light beam. We describe the principle of operation of the laser system, which is generally based on a wavelength-tunable alexandrite laser oscillator / amplifier, and multiple Raman conversion stages. This configuration allows for robust access to the mid-IR spectral region at wavelengths, pulse energies, pulse durations, and repetition rates that are attractive for neurosurgical applications. We report results for ultra-precise selective cutting of nerve sheaths with little collateral damage, which has applications in procedures such as optic-nerve-sheath fenestration and possible spinal repair. We also report results for cutting cornea and dermal tissues. We review design factors that impact the ease of use and integration in a clinical setting, including beam delivery systems and the use of diode pumping to reduce system size and limit downtime.

8928-27, Session PSat

Development of a fiber-less fNIRS system and its application to hair-covered head

Toru Yamada, National Institute of Advanced Industrial Science and Technology (Japan); Mitsuo Ohashi, Spectratech Inc. (Japan); Shinji Umeyama, National Institute of Advanced Industrial Science and Technology (Japan)

While most commercially available fNIRS systems have used optical fibers for the measurement optode and the transmission cable of the optical signals, their material inflexibility is not very suitable for a stable optode fixation to the head surface and an adequate cable lining to the main system. In practice, mechanical fluctuations of such fibers often have led motion artifacts to the fNIRS signals. A few commercial systems equip with light sources and detectors adhering directly to scalp surface; however, both the shapes and sensitivities of such optodes were not applicable for the usage over a hair-covered head. Based on a commercial fiber-less fNIRS system OEG-16 (Spectratech, Inc., Japan), we developed a new optode unit which was designed with LED of enhanced illumination, APD changed from PD, and a new holder system. The electrical circuits of system were modified after the design. By simultaneous implementations of the multidistance fNIRS measurement and hemodynamic modality separation for conventional fNIRS data at bilateral parietal area during single-sided motor tasks, significant functional signals were observed at the position contralateral to the side of movement in both cases. This is the first report that a fiber-less fNIRS system could detect functional signals at hair-covered head. We believe this fiber-less system gains fNIRS utility especially in the less restrained condition.

8928-28, Session PSat

Precise spatial co-registration in simultaneous fNIRS and fMRI measurements using markers coaxially fixable to the optodes

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As well as the blood oxygenation level dependent (BOLD) MRI, the functional near-infrared spectroscopy (fNIRS) observes a regional hemodyanamic response associated with neuronal activation. However, the conventional criteria for detecting fNIRS signals and BOLD signals do not seem to stand on a unified understanding on the cerebral hemodynamics. Not a few fNIRS studies ascribed an increase in oxy-hemoglobin to the typical functional hemodynamics while the BOLD signal in MRI directly correlates with a decrease in deoxy-hemoglobin. Such inconsistency needs to be solved through the simultaneous measurement of fNIRS and MRI. In practice, however, there remain several technical problems to conduct it with high reproducibility. One of the issues is a precise spatial registration of NIRS optode in the structural or functional images in MRI. We prepared marker containers of toroidal shape that can be coaxially fixed to optodes. Liquid paraffin containing ?-tocopheryl acetate, which indicates a bright contrast in T1-weighted MR images of human head, was solidified in each container by adding higher fatty acid. A subject wearing the fNIRS optodes with markers at his parietal area participated a preliminary experiment; the subject was instructed to execute single-sided hand finger tapping. The superposition of structural and functional MR images clearly showed the positional relationship between the measurement channel and activated region. The marker prepared is chemically stable and repetitively usable. We believe that this simple method gains the precision in co-registration of fNIRS and fMRI.

8928-29, Session PSat

Fluorescence micro-optical sectioning tomography (fMOST)

Xiaoli Qi, Britton Chance Ctr. for Biomedical Photonics (China)

Revealing neural circuit mechanisms is critical for understanding brain functions. Significant progress in dissecting neural connections has been made using optical imaging with fluorescence labels, especially in dissecting local connections. However, acquiring and tracing brain-wide, long-distance neural circuits at the neurite level remains a substantial challenge. Here, we develop an automated fluorescence micro-optical sectioning tomography system for long-term stable imaging. With the unprecedented ability to image a whole mouse brain at a one-micron voxel resolution. Our method is believed to open an avenue to exploring both local and long-distance neural circuits that are related to brain functions and brain diseases down to the neurite level.

8928-30, Session PSat

Photothermal therapy of human glioma spheroids with gold-silica nanoshells and gold nanorods: a comparative study

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Gold-based nanoparticles including gold-silica nanospheres and gold nanorods have been investigated for a number of therapeutic and diagnostic applications. The ability of these nanoparticles to convert light into heat energy makes them particularly appealing for photothermal therapy in which cancer cells are destroyed via light-induced heat generation. The overall objective of the study was to compare the efficacy of gold-silica nano-spheres and gold nano-rods in an in vitro system consisting of human brain tumor (glioma) spheroids.

Delivery of nanoparticles to the spheroids was accomplished using murine macrophages. Nanoparticles (spheres or rods) were incubated with macrophages for 24 hours. Thereafter, nanoparticle-loaded macrophages were combined with human glioma cells and centrifuged in order to create hybrid spheroids. Approximately 48 hours post-centrifugation, the resultant 400 µm dia. spheroids were exposed to 808 nm laser light for 10 min at irradiances of 2, 7, 14 and 28 W cm-2. Treatment efficacy was evaluated from spheroid growth kinetics over a 14-day period.

Gold nanoshells were shown to have greater efficacy compared to gold nanorods. For example, hybrid spheroids consisting of a 5:1 ratio of glioma cells to nanosphere-loaded macrophages exhibited significant growth inhibition when subjected to irradiances of 7 W cm-2. In contrast, no growth inhibition was observed for the nanorod-macrophage hybrid spheroids, even at the highest irradiance investigated (28 W cm-2). Growth inhibition was observed at 28 W cm-2 when the nanorod concentration was increased, i.e., by forming hybrid spheroids with a 2:1 ratio of glioma cells to macrophages.

8928-31, Session PSat

Activity-dependent signal changes in neurons by fiber-coupled microscopy

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By using the fiber-coupled microscope (FCM), in vivo imaging is possible at the deep site in the tissues or organs where other optical techniques are difficult to reach. The optical performance of the FCM highly depends on the properties of the fiber bundle which detects an emitting light from cells. However, the quality and resolution of the images obtained by the conventional FCM was low to examine the neuronal signals. To improve the spatial resolution of the FCM, we used a high density type of the imaging fiber (HDI) which included 15000 unit fibers and each diameter was less than 3 ?m. The HDI composed a circular field of view of about 300 ?m wide, thus spatial resolution was 2.3 ?m/unit on average. The tip of the HDI was tangentially trimmed and either an optical lens was attached (gradient index lenses; GRIN fiber) or not (bare fiber). Total diameter of the HDI was less than 450 ?m in either case. The HDI we employed here produced a spatial-resolution 1.5 fold higher than the conventional fibers we used before. The activity-dependent signals of each neuron were successfully detected from in vitro preparation of the rodents' cortex by using the HDI with a fluorescence imaging system consisting of laser, objective lens and CCD camera. The intracellular details of fluorescence pattern and their temporal changes, difficult to observe with the former system, were easily detected by using the new FCM system. We also observed the intrinsic signals of individual neurons which reflected metabolic states of the cells and found that the HDI was capable as realtime (10 frames/s) label-free cellular imaging probes. These fibers can penetrate deeper than 1 mm from the surface of the cortex. The potential power of the FCM will be expected to rise continuously for the analysis of a fine structure of the cells and an organization of certain functions in vivo.

8928-32, Session PSat

Multimodal intraoperative optical spectroscopy probe for tissue characterization during brain tumor resection

Jeanne Mercier, Karl St-Arnaud, Liane Bernstein, Yoann Gosselin, Jean-David Grenon, Audrey Laurence, Ecole Polytechnique de Montréal (Canada); Pablo A. Valdes, Dartmouth College (United States); Kelvin Mok, Mohamad Seyed Sadr, Kevin Petrecca, Montreal Neurological Hospital and Institute (Canada); Frédéric Leblond, Ecole Polytechnique de Montréal (Canada)

Complete but selective tumor removal during neurosurgical procedures is a matter of great importance for the patient's prognosis. Remaining cancerous cells may jeopardize the patient's remission, which is why an accurate and quantitative technique for detecting tumor boundaries is essential. Here, we used an innovative multimodal intraoperative optical spectroscopy probe to characterize tissues using inelastic Raman scattering combined with fluorescence and reflectance.

The results of in vivo experiments evaluating the accuracy of tumor detection using this new technique are presented herein. Xenograft glioma tumor cells expressing green fluorescent protein (GFP) were implanted in immunodeficient mice on which a craniotomy was performed, as well as on controls. With the purpose of detecting tumor margins, both GFP and aminolevulinic acid-induced protoporphyrin IX fluorescence were imaged using a wide-field hyperspectral fluorescence detection system ensuing tissue excitation. A series of measurements was concurrently made with the probe, then all spectra were classified as within or outside these tumor margins with statistical methods. Subsequently, the results were compared with wide-field studies and histology. Result analysis shows that our novel setup provides quick and reliable information about the tissue's nature which can help guide the surgeon through an efficient tumor resection.

These results are all the more encouraging as they suggest that this technique could be used to replace random biopsies of suspicious tissues along with reducing time, fluctuations, and costs of the procedure. It could also potentially be generalized to other types of tumor such as those found in the throat or prostate.

8928-33, Session PSat

Frontal activation during verbal fluency test using diffuse optical probes

Jing Dong, See Khee Ng, Renzhe Bi, Kijoon Lee, Nanyang Technological Univ. (Singapore)

Diffuse optical spectroscopy (DOS) and diffuse correlation spectroscopy (DCS) are novel brain study techniques in recent years. These non-invasive methods use wavelength in the near-infrared light range to measure hemodynamic response from diffusely scattered light. Particularly, DOS allows the measurements of oxy- and deoxy-hemoglobin concentration, tissue oxygen saturation and total hemoglobin concentration while DCS provides the information of blood flow.

In order to study the coupling relationship of cerebral hemodynamic response and verbal fluency task (VFT), we designed a probe incorporated both diffuse optical and correlation spectroscopy and secured on left frontal cortex by a self-made headband. There were totally 12 normal, healthy and right-hands subjects (24±1 year-old) participated the study. The protocol consisted of alternating rest and excitation stages. In rest period, the subject was given a set of numbers from 1 to 100 to recite aloud to induce the jaw movement whereas in excitation periods (verbal fluency task), the alphabet “F” “A” and “S” were given in turn.

Results from the experiment were analyzed by examining the patterns to deduce the brain functional state of the subject. Generally, HbO2 increased while Hb decreased during the task period, but no trends of rBF were detected when comparing rest and excitation phases. Statistical analysis of HbO2 and Hb between rest and excitation stages showed significant difference (p=5.38E-6 and p=8.85E-8, respectively), but not for rBF (p=0.438). Through the NIRS-SPM time series analysis, we found the correlation between HbO2, Hb response and VFT. It suggests the hemodynamic response of VFT may originate from the skin besides frontal cortex.

8928-34, Session PSat

Targeted principle component analysis: a new motion artifact correction approach for near-infrared spectroscopy

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As Near-Infrared Spectroscopy (NIRS) broadens its application area, motion artifacts in the NIRS signal due to subject movement is becoming an important challenge. Motion artifacts generally produce signal fluctuations that are larger than physiological NIRS signals, thus it is crucial to correct for them before obtaining an estimate of stimulus evoked hemodynamic responses. There are various methods for correction such as principle component analysis (PCA), wavelet-based filtering and spline interpolation. Here, we introduce a new approach to motion artifact correction, targeted principle component analysis (tPCA), which incorporates a PCA filter only on the segments of data identified as motion artifacts. We compared the new approach with the most effective motion artifact correction algorithms on a set of data acquired simultaneously with a collodion-fixed probe (low motion artifact content) and a standard Velcro probe (high motion artifact content). Our results show that tPCA gives statistically better results in recovering HRF as compared to wavelet-based filtering and spline interpolation for the Velcro probe. It results in a significant reduction in mean-squared error and significant enhancement in Pearson’s correlation coefficient to the true HRF. The collodion-fixed fiber probe with no motion correction performed better than the Velcro probe corrected for motion artifacts in terms of mean-squared error and Pearson’s correlation coefficient. Thus, if the experimental study permits, the use of a collodion-fixed fiber probe may be desirable. If the use of a collodion-fixed probe is not feasible, then we suggest the use of tPCA in the processing of motion artifact contaminated data.

8928-13, Session 4

5-ALA based photodynamic management of glioblastoma

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Since fluorescence guided resection (FGR) with 5-aminolevulinic acid (5-ALA) has become available, only few groups have explored the potential of photodynamic therapy (PDT) based on 5-ALA induced Protoporphyrin IX (PpIX) for the treatment of glioblastoma. In most cases, Photofrin was used in addition, not trusting in a sufficient phototoxic potential of the accumulated PpIX. We have performed a controlled study and several individual treatment attempts to apply interstitial 5-ALA-PDT with stereotactically implanted radial diffuser fibers. From quantitative assessments of PpIX tumor concentrations, we learned that PpIX concen-trations can vary extremely from patient to patient. We have therefore recently implemented PpIX-fluorescence measurements during the interstitial PDT irradiation. For this purpose, one of the diffuser fibers is connected to a spectrometer, recording the emission of the PpIX peak at 705 nm, while one of the others irradiates the tissue with the treatment wavelength.

The observed photobleaching was further examined as a potential treatment monitoring tool. Monte Carlo computer simulations showed that it might be possible to predict the required irradiation time in a given configuration of radial diffusers by monitoring the fluorescence signal during PDT irradiation. The time-dependent fluorescence decay depends characteristically on absorption and scattering within the irradiated tissue volume, and the ratio of fluorescence signals at different points of time indicates the required light dose for an efficient photobleaching throughout the irradiated volume.

A better individualization of PDT protocols might improve treatment outcomes. Currently, we can at least predict potential responders and have achieved recurrence-free survival times of 29, 30 and 36 months among 5 patients treated.

8928-14, Session 4

Ultra-low fluence rate photodynamic therapy: simulation of light emitted by the Cerenkov effect

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PDT has been shown to be most effective at low fluence rates. Many radionuclides used for both diagnostic and therapeutic purposes produce measurable amounts of visible radiation when they decay via the Cerenkov effect which occurs when a charged particle travels faster in a dielectric medium than the speed of light that medium. Cerenkov

radiation from radiopharmaceuticals could serve as a source of extended duration, low level “internal” light, to mediate PDT, with the ultimate goals of overcoming some its current limitations. Using laser light, we are exploring the effects of fluence rates that could be generated by Cerenkov radiation, on PDT efficacy.

ALA or TPPS2a mediated PDT of rat glioma monolayers or multicell spheroids (F98, C6) was performed with 410 nm laser light exposure over an extended period of 24-72hrs. Photosensitizers were delivered either as a bolus or continuously with light exposure. At fluence rate of 20?W/cm2 effective PDT was obtained as measured by decrease in cell viability or inhibition of spheroid growth. We are presently evaluating lower fluence rates over longer time periods.

PDT is effective at ultra low fluence rates if given over long time periods. No lower threshold has been ascertained. Since the half-life of 90Y, a radionuclide with a high Cherenkov yield is 64 hrs it is a good candidate to supply sufficient light activation for PDT. The combination of radionuclide and photodynamic therapies could improve the effectiveness of cancer treatment by exploiting synergies between these two modalities.

8928-15, Session 4

Integrated optical spectroscopy system to guide brain needle biopsies

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In order to provide more sensitive and specific pathological information on brain tumors than available with conventional imaging techniques, stereotactic brain needle biopsies (BNB) are performed. This can be a hazardous procedure due to the risk of clipping blood vessels leading to hemorrhaging, while false diagnosis can result when the collected sample is not representative of the tumor. Here, we offer an innovative technology that can help mitigate both these risks. By integrating a multimodal optical spectroscopy system to the biopsy needle, it would be possible to differentiate normal from cancerous cells as well as to locate blood vessels nearby. We developed a novel tomographic approach for imaging with diffuse light using a probe made of several evenly distributed fibers that permit a 360-degree reconstruction of tissues adjacent to the probe. Using our first prototype based on this approach, we have demonstrated its ability to identify high absorbers, such as blood vessels, located up to 2 mm away from the probe surface. By doing so, this imaging technique considerably reduces the risks of BNB-related hemorrhages. Furthermore, the same prototype performs fluorescence measurements of aminolevulinic acid induced protoporphyrin IX (PpIX), an accurate biomarker of cancerous cells in the brain. Quantifying the concentration of PpIX allows for a reliable identification of the tumor location, thus eliminating the need for multiple biopsies. Future work will involve the evaluation of this technology in terms of safety and accuracy improvements in the scope of a clinical study.

8928-16, Session 4

Quantitative optical-sectioning microscopy of 5-ALA-induced PpIX in human low-grade gliomas

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Extent of resection (EOR) has been shown to be a major predictor of glioma patient outcomes. While wide-field imaging of 5-ALA-induced PpIX has been shown to improve EOR for high-grade gliomas, it lacks the resolution and sensitivity to detect PpIX in low-grade gliomas (LGG), where PpIX expression is sparse. Here we investigate the potential of high-resolution optical-sectioning microscopy to quantify PpIX expression in LGGs with high sensitivity and accuracy. In order to assess the feasibility of this quantitative imaging technique, we compare the accuracy of dual-axis confocal (DAC) microscopy-based quantification of PpIX expression vs. histological PpIX quantification in fixed human LGG tissue samples. A robust image-processing algorithm that is relatively independent of detector gain settings has been developed to quantify the density of PpIX expression in LGG tissue samples with both imaging techniques. The accuracy of PpIX quantification exhibited by DAC microscopy supports its clinical utility and suggests that intraoperative optical-sectioning microscopy could potentially serve as a valuable adjunct to wide-field imaging techniques for guiding the surgical resection of LGGs.

8928-17, Session 5

Combining ICA and granger causality: a novel tool for investigation of brain dynamics and brain oscillations using fNIRS measurements

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Granger causality mapping (GCM) derived from vector autoregressive models of data is able to reveal complex temporal and spatial dynamics underlying cognitive processes. However, the traditional GCM methods are computationally expensive, as signals from thousands of voxels are individually processed. Additionally functional near infrared spectroscopy (fNIRS) is a non-invasive method to capture brain activity according to the measurement of changes in both oxyhemoglobin and deoxyhemoglobin concentration. However, fNIRS recordings are the hemodynamic signals that come from the latent neural sources that are spatially and temporally mixed across the brain.

In this study a new algorithm called ICA based GCM is proposed to overcome these problems. The algorithm implements the following two procedures: (i) extraction of the temporal time courses and the spatial activation mapping of region of interests (ROIs) by independent component analysis (ICA), and (ii) estimation of the direct causal influences in local brain networks using Granger causality and independent time courses of ROIs. Our results show the use of ICA in conjunction with GCM are able to effective identify the ROIs and greatly reduces the computational cost relative to the use of individual voxel time series for capturing the brain dynamics and brain oscillations in neural network analysis.

8928-18, Session 5

Monitoring closed head injury induced changes in brain physiology with orthogonal diffuse near-infrared reflectance spectroscopy

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We applied an orthogonal diffuse reflectance spectroscopy (o-DRS) to assess brain pathophysiology following closed head injury (CHI). CHI usually caused by mechanical blows to the head occurs in traffic accidents, falls and assaults. In this study, CHI was induced in anesthetized male ICR mice by weight-drop method using ~50gram cylindrical metal falling from a height of 90 cm onto the intact scalp.

A total of 26 mice (aged: ~12 weeks, weight: ~40 g) were used in the experiments divided randomly into three groups as follows: Group 1 (n=11) consisted of injured mice monitored for 1 hour every 10 minutes. Group 2 (n=10) were the control mice not experience CHI. Group 3 (n=5) consisted of injured mice monitored every minute up to 20 minutes. Measurement of optical quantities of brain tissue (absorption and reduced scattering coefficients) in the near-infrared from 650 to 1000 nm were carried out by employing different source-detector distances and locations to provide depth sensitivity. With respect to baseline, we found difference in brain hemodynamic properties following injury. In addition, the o-DRS also successfully evaluated the structural variations likely from evolving cerebral edema throughout exploring the scattering spectral shape. In total, experimental results show the potential use of the proposed technique for monitoring brain function following injury.

8928-19, Session 5

Near-infrared diffuse reflectance imaging of infarct core and peri-infarct depolarization in a rat middle cerebral artery occlusion model

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To understand pathophysiology of ischemic stroke, in vivo imaging of brain tissue viability and related spreading depolarization is crucial. In the infarct core, impairment of energy metabolism causes anoxic depolarization (AD), which considerably increases energy consumption, accelerating irreversible neuronal damage. In the peri-infarct penumbra region, where tissue is still reversible in spite of limited blood flow, peri-infarct depolarization (PID) occurs, exacerbating energy deficit and hence expanding the infarct area. We previously showed that light-scattering signal, which is sensitive to cellular/subcellular structural integrity, was correlated with AD and brain tissue viability in a rat hypoxia-reoxygenation model (Kawauchi, et al., JBO 2013). In the present study, we performed transcranial NIR diffuse reflectance imaging of the rat brain during middle cerebral artery (MCA) occlusion and examined whether infarct core and PIDs can be detected by measuring NIR reflectance. Immediately after occluding the left MCA, light scattering started to increase focally in the occlusion site and a bright region followed by a dark region was generated near the occlusion site and spread over the left entire cortex. The wave (PID) was generated repetitively; the number of times of PID in one rat ranged from four to ten within 1 hour after occlusion. The scattering increase in the occlusion site was irreversible and the area with increased scattering expanded with increasing the number of PIDs, which may indicate expansion of the infarct core. These results suggest the usefulness of NIR diffuse reflectance signal to visualize spatiotemporal changes in infarct area and PIDs in focal ischemia.

8928-20, Session 5

Implantable CMOS imaging device with absorption filters for green fluorescence imaging

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Green fluorescent materials such as GFP (Green Fluorescence Protein) and fluorescein are often used for observation of brain neural activities. It is important to observe those of fluorescence in freely moving state to understand neural activities corresponding to behaviors. In this work, we developed an implantable CMOS imaging device for in-vivo green fluorescence imaging with efficient excitation light rejection by combination of absorption filters.

An interference filter is usually used for a fluorescence microscope in order to achieve high fluorescence imaging sensitivity. However, in the case of our implantable imaging device, interference filters are not suitable, because the device mounts no imaging optics and thus fluorescent light incidents on the device in any angles, which degrades the filter performance. To solve this problem, we used two kinds of absorption filters, which have non-angle dependence.

An absorption filter made of yellow dye (VARYFAST YELLOW 3150) was coated on the pixel array of an image sensor. The rejection ratio of ideal excitation light (490 nm) against green fluorescence (510 nm) was 99.66 %. However, the blue LED as an excitation light source has a broad emission spectrum and its intensity at 510 nm is 2 % of the emission peak. By coating the excitation filters, the unwanted component of the excitation light intensity was reduced to 0.004 %.

By the combination of the two absorption filters, we achieved excitation light transmittance of 10-5 through the filter on the image sensor. It is expected that this technology enables green fluorescence imaging of neural activities with high sensitivity in the freely moving mouse.

8928-21, Session 5

Time-resolved measurement and imaging reconstruction validated by a realistic dynamic optical brain phantom

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Diffuse optical tomography (DOT) is a sensitive and relatively low cost technique based on the propagation of near-infrared (NIR) light in a scattering dominant medium. DOT can be used to visualize hemodynamic responses and reconstruct optical properties of human brain tissue by measuring changes in optical absorption of NIR light. We used a fast time domain DOT system to image a realistic dynamic optical human brain phantom. The optical properties of the brain phantom were modulated to mimic the arterial blood flow in brain. Changes in the recorded optical signals corresponded to changes in the optical properties of the brain phantom. Moreover, these time-dependent optical signals were used to reconstruct the dynamic optical property changes with a novel image reconstruction algorithm. This algorithm is based on dynamic perturbation theory, combining a priori structure information and a linear pseudoinverse method. The experiment results confirm that the new algorithm reliably reconstructs the time-dynamic optical inhomogeneities. In addition, the reconstruction validity was also investigated with different distributions of inhomogeneities.

8928-22, Session 5

In vivo imaging of scattering and absorption properties of exposed brain using a digital red-green-blue camera

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We investigate a method to estimate the spectral images of reduced scattering coefficients and the absorption coefficients of in vivo exposed brain tissues in the range from visible to near-infrared wavelength (500-760 nm) based on diffuse reflectance spectroscopy using a digital RGB camera. In the proposed method, the multi-spectral reflectance images

of in vivo exposed brain are reconstructed from the digital red, green blue images using the Wiener estimation algorithm. The Monte Carlo simulation-based multiple regression analysis for the absorbance spectra is then used to specify the absorption and scattering parameters of brain tissue. In this analysis, the concentration of oxygenated hemoglobin and that of deoxygenated hemoglobin are estimated as the absorption parameters whereas the scattering amplitude a and the scattering power b in the expression of musp=a(lambda)^-b as the scattering parameters, respectively. The spectra of absorption and reduced scattering coefficients are reconstructed from the absorption and scattering parameters, and finally, the spectral images of of absorption and reduced scattering coefficients are estimated. A white light emitted diode illuminated the cortical surface via a light guide with a ring-shaped illuminator. The RGB images of the exposed cortex were acquired by a 24-bit RGB CCD camera without an IR cut filter. The estimated images of absorption coefficients were dominated by the spectral characteristics of hemoglobin. The estimated spectral images of reduced scattering coefficients showed a broad scattering spectrum, exhibiting larger magnitude at shorter wavelengths, corresponding to the typical spectrum of brain tissue published in the literature.

8928-23, Session 6

Hemodynamic measurements in deep brain tissues of humans by near-infrared time-resolved spectroscopy

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To measure various optical properties and hemodynamics of deep brain tissues of humans, we developed a highly sensitive near-infrared time-resolved spectroscopy (TRS) system, whose algorithm utilizes the wavelength-dependence of the reduced scattering coefficient. This highly sensitive TRS system consists of high-power light sources called Nanosecond Light Pulsers, a photomultiplier tube (PMT) for single-photon counting, a TRS circuit based on the time-correlated single-photon counting method, and optical fibers. We used the optical detection fiber with a numerical aperture of 0.56, and a highly-sensitive PMT with a GaAs photocathode. Using this system, we measured the optical properties and hemodynamics of deep brain tissues in 50 healthy adult volunteers. The right ear canal was irradiated with a 3-wavelength pulsed light (760, 795, and 835 nm), and the photons passing through the brain tissue were collected at the left ear canal. Optical signals with sufficient intensity were obtained from 46 of these 50 volunteers. By analyzing the temporal profiles based on photon diffusion theory, we successfully obtained reduced scattering and absorption coefficients for each wavelength. Thereafter, we determined the levels of oxygenated hemoglobin, deoxygenated hemoglobin, total hemoglobin, and tissue oxygen saturation by referring to the hemoglobin spectroscopic data. Compared to the oxygen saturation values for the forehead measurements, the values from the deep brain tissues were approximately 10% lower. Moreover, total hemoglobin concentrations of deep brain tissues were always lower than those of the forehead measurements. These results demonstrated a potential application of this TRS system in examining deep brain tissues of humans.

8928-24, Session 6

Laplace-domain diffuse optical brain imaging system

Ali Hasnain, Kalpesh Mehta, Xiaowei Zhou, Nanguang Chen, National Univ. of Singapore (Singapore)

Diffuse optical imaging (DOI) is an emerging modality for imaging biological tissues with greater penetration depth, improved specificity and the ability to provide spatiotemporal functional information. DOI generates optical contrast from near-infrared light propagation through scattering tissues and absorption by main chromophores of tissue; dominantly oxy-hemoglobin (HbO) and deoxy-hemoglobin (HbR). This enables DOI to monitor hemodynamic changes related to cerebral activation. We have developed a novel Laplace-domain diffuse optical tomography (DOT) system for imaging brain functions. The system uses high speed laser diodes modulated with 2.5GHz pseudo-random bit sequence (PRBS) for tissue illumination and time-resolved measurements are retrieved by cross-correlating the reflected signal from tissue with the reference PRBS. The resuting signal is then directly harware-transformed into Laplace domain to extract featured data sets. The unique architecture of our system allows to image at a much higher frame rate as compared to the conventional time-domain DOT systems, makes it low cost and compact than most frequency domain DOT systems and less computationally exhaustive for tomographic reconstruction. 80-optode pairs, over two distinct wavelengths on either sides of isosbestic point, are used to monitor cortical hemodynamic activity. This leads the system to simultaneously record changes in the concentration of HbO and HbR and the total hemoglobin concentration (HbT) with good temporal resolution during different motor tasks. In this work, we will be presenting our novel system with preliminary results and comparisons of multiple cortical activities using different stimuli.

8928-26, Session 6

Noninvasive optical evaluation of low frequency oscillations in prefrontal cortex hemodynamics during verbal working memory

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The low frequency oscillation (LFO) around 0.1 Hz has been observed recently in cerebral hemodynamic signals during rest/sleep, enhanced breathing, and head- up-tilting, showing that cerebral autoregulation can be accessed by LFOs. However, many brain function researches require direct measurement of LFOs during specified brain function activities. This pilot study explored using near-infrared spectroscopy/ imaging (NIRS) to noninvasively and simultaneously detect LFOs of prefrontal cerebral hemodynamics (i.e., oxygenated/deoxygenated/total hemoglobin concentration: ?[oxy-Hb]/ ?[deoxy-Hb]/ ?[tot-Hb]) during N-back visual verbal working memory task. The LFOs were extracted from the measured variables using power spectral analysis. We found the brain activation sites struck clear LFOs while other sites did not. The LFO of ?[deoxy-Hb] acted as a negative pike and ranged in (0.05, 0.1) Hz, while LFOs of ?[oxy-Hb] and ?[tot-Hb] acted as a positive pike and ranged in (0.1, 0.15) Hz. The amplitude difference and frequency lag between ?[deoxy-Hb] and ?[oxy-Hb]/ ?[tot-Hb] produced a more focused and sensitive activation map compare to hemodynamic amplitude-quantified activation maps. This study observed LFOs in brain activities and showed strong potential of LFOs in accessing brain functions.

8928-35, Session 7

Infrared light can block onset responses to kilo hertz frequency nerve block *(Invited Paper)*

Emilie H. Lothet, Kevin Kilgore, Niloy Badhra, Narendra Badhra, Tina Vrabec, Yves T. Wang, Case Western Reserve Univ. (United States); E. Duco Jansen, Vanderbilt Univ. (United States); Michael W. Jenkins, Hillel Chiel, Case Western Reserve Univ. (United States)

Nerve block can reduce or even eliminate spasms and chronic pain. Kilo hertz frequency alternating current (KHFAC) produces safe and reversible nerve block. However, KHFAC-induced nerve block is preceded by an undesirable onset response. Optical inhibition (OI) can produce nerve block without an onset response, but heat deposition may be deleterious to the nerve over long times. Combining KHFAC with OI could produce a safe nerve block without an onset response that could be used for an extended period of time.

Unmyelinated Aplysia nerves were used. A monopolar suction electrode was used to deliver the test stimulation and bipolar suction electrodes were used for the proximal, blocking and distal electrodes. Two Aculight Capella infrared lasers were coupled through 600-µm multimode fibers and both were angled at 30 degrees at a single point on the nerve between the blocking and distal electrode. The KHFAC block threshold was found. Lasers were turned on before KHFAC to induce an effective temperature change in the nerve.

The onset response was successfully eliminated by combining KHFAC and OI. Reversible block was established with 30 seconds of OI with 0.015-0.023 J/cm2 per pulse before the KHFAC. The KHFAC block at 10 kHz was usually obtained at a current of 15 mA. OI was also used to block action potentials in under 300 msec. No obvious changes in the physiology of the nerve were observed.

Safe and reversible nerve block without an onset response was observed by combining KHFAC and OI.

8928-36, Session 7

Imaging of injury and recovery in experimental models of brain injury using intrinsic scattering signatures *(Invited Paper)*

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

The penumbra is an area of brain tissue which is compromised during acute stroke but may possibly be salvaged. Unless perfusion is improved through thrombolysis, or cells are made more resistant to injury within hours, the penumbra dies as the infarct core expands over time. We present a multi-parametric OCT imaging approach that provides the capability to simultaneously image the interplay of absolute blood flow, cell viability, and vascular remodeling, factors that are of interest in determining the fate of the penumbra, in brains of mice, with high spatiotemporal resolution. Longitudinal imaging was demonstrated during both progression in the acute stage and repair in the chronic stage.

In an acute stroke model, a striking pattern of capillary non-perfusion was imaged during occlusion. Eventually, this non-perfused region exhibited alterations in scattering properties, despite capillary reperfusion following filament withdrawal. Histological correlations showed that the altered scattering properties may reflect aberrant cellular morphology that is characteristic of infarct tissue. Vessel dilation and increased blood flow were also noted in the middle cerebral artery (MCA) region following reperfusion. This “luxury perfusion” may at first appear paradoxical, but can potentially be explained by failure of autoregulation in the region destined for infarction. In addition, chronic vascular remodeling in a permanent distal MCA occlusion model was investigated. Our imaging results suggest capillary non-perfusion, altered cellular scattering, flow deficits, and impaired flow autoregulation as candidate biomarkers for eventual tissue infarction. Thus, OCT represents a novel imaging platform for longitudinally mapping hemodynamics and cell status during injury and recovery in cerebrovascular disease.

8928-37, Session 7

Towards holographically-patterned three-dimensional neuronal stimulation in a bioengineered optonet *(Invited Paper)*

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Planar neural networks and interfaces serve as a versatile in vitro model of CNS physiology, but adaptations of related methods to three dimensions (3D) have met with limited success. Although early attempts at extending MEA technology to 3D were demonstrated, it is generally accepted that opto-physiology methods are optimally suited for the required non-contact, volumetric interfacing. Suitable neuro-photonic interfaces will require display methods that can selectively excite across large neuronal populations.

Recently, we demonstrated holographic projection as a powerful excitation strategy in conjunction with optogenetic stimulation or photo-absorber induced neural-thermal stimulation (PAINTS) for efficiently, selectively and predictably controlling large neuronal populations, with milisecond temporal precision, and cellular specificity.

Here we explore the use of holographic projection based on phase-SLMs as a strategy for volumetric targeted photo-stimulation in an optically accessible 3D neuronal network ‘optonet’. These consist of rat primary cortical cells densely embedded in a transparent hydrogel scaffold which facilitates the growth and development of neural cells into networks with brain-like density and composition. We further explore the integration of the holographic stimulation modules with additional components including piezo-based 3D microscopes for functional imaging of distributed network activity patterns and an acoustic transducer for opto-acoustic localization of the dark micro-particles used for PAINTS.

These expand the applicability of high-rate holographic projection as an enabling photo-stimulation tool in neural interfaces with high spatial-temporal precision, and open up a new range of applications based on optonets as a versatile 3D in vitro tool for drug screening and regenerative medicine.

8928-38, Session 7

Optical pacing of developing hearts

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No Abstract Available

8928-39, Session 7

Laser-acupuncture for autism/autism spectrum disorder: a randomized sham controlled trial

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OBJECTIVES: To evaluate the efficacy, safety, and compliance of laser-acupuncture in children with autism spectrum disorder (ASD). DESIGN: Randomized, sham controlled, single blind trial, with blinded evaluation and statistical analysis of results. SUBJECTS AND INTERVENTIONS: Children with ASD were randomly separated into two groups one receiving laser-acupuncture (LA) group (n=60) and the other sham laser-acupuncture (SLA) group (n=56) matched by age and severity of autism. The LA group received laser-acupuncture for selected acupoints while the SLA group received sham laser-acupuncture to sham acupoints. A total of 24 LA and SLA sessions over 12 weeks were given. Primary outcome measures included Functional Independence Measure for Children (WeeFIM), Pediatric Evaluation of Disability Inventory (PEDI), Leiter International Performance Scale- Revised (Leiter-R), and Clinical Global Impression- Improvement (CGI-I) scale. Secondary outcome measures consisted of Aberrant Behavior Checklist (ABC), Ritvo-Freeman Real Life Scale (RFRLS), Reynell Developmental Language Scale (RDLS), and a Standardized Parental Report. Data were analyzed by the Mann-Whitney test. RESULTS: There were significant improvements in the language comprehension domain of WeeFIM (p=0.02), self-care caregiver assistant domain of PEDI (p=0.028), and CGI-I (p=0.003) in the LA group compared to the SLA group. As for the parental report, the LA group also showed significantly better social initiation (p=0.01), receptive language (p=0.006), motor skills (p=0.034), coordination (p=0.07), and attention span (p=0.003). All children with ASD adapted to laser-acupuncture easily. Mild side effects of irritability during laser-acupuncture were observed. CONCLUSION: A twelve-week (24 sessions) course of laser-acupuncture is useful to improve specific functions in children with ASD, especially for language comprehension and self-care ability.

8928-40, Session 8

Studying hemispheric lateralization during a Stroop task by near-infrared spectroscopy *(Invited Paper)*

Lei Zhang, Hui Gong, Huazhong Univ. of Science and Technology (China)

Local brain activity induces an increase in local cerebral blood volume (CBV) and blood flow (CBF) termed neurovascular coupling. Near-infrared spectroscopy (NIRS) is a noninvasive optical method that takes advantage of the sensitivity of NIR light to hemoglobin oxygenation-state shifts. In this study, we measured hemodynamic activity of the prefrontal cortex (PFC) during a Chinese color-word matching Stroop task using a homemade continuous-wave NIRS imaging system. During the experiment, the subjects were asked to examine the consistency of the color of the upper character and the meaning of the lower character in the screen and press the corresponding button as quickly as possible. Two types of blocks, i.e. neutral stimuli block and incongruent stimuli block were presented in rotation during the Stroop task. Two NIRS probes were placed separately over the left and the right PFC. Each probe contained of one source and eight detectors to provide eight detector channels. Wavelet transform coherence (WTC) analysis was employed to calculate coherences between all channels of the same probe pairwise to obtain the intrahemispheric functional connectivity for each side of the PFC. The results shown that, the intrahemispheric functional connectivities in both sides of PFC were stronger during the incongruent stimuli block compared to that of the neutral stimuli block, but only the left intrahemispheric functional connectivity showed a significant Stroop effect. Our findings demonstrate that NIRS-based functional connectivity is a valuable tool for studying hemispheric lateralization in high-level and complex cognitive tasks.

8928-41, Session 8

Quantitative assessment of brain tissue oxygenation in porcine models of cardiac arrest and cardiopulmonary

resuscitation using hyperspectral near-infrared spectroscopy *(Invited Paper)*

Shahin S. Lotfabadi, Ryerson Univ. (Canada)

Near-infrared spectroscopy (NIRS) is a powerful tool to measure real-time tissue oxygenation in the brain. We used a broad-band, continuous-wave hyper-spectral approach to measure tissue oxygenation in the brain of piglets under the conditions of cardiac arrest, cardiopulmonary resuscitation (CPR), and defibrillation. The purpose of this research was to find a correlation between mortality due to cardiac arrest and inadequacy of the tissue perfusion during attempts at resuscitation. Using this technique we measured the changes in concentrations of oxy-hemoglobin and deoxy-hemogolobin to quantify the tissue oxygenation in the brain. We also extracted cytochrome c oxidase changes under the same conditions to determine increase or decrease in cerebral oxygen delivery. In this paper we proved that applying CPR, oxy-hemoglobin concentration and brain tissue oxygenation in the brain increase while deoxy-hemogolobin concentration decreases which was not possible using other measurement techniques. We concluded that improving CPR methodology could increase the patient survival chance after a cardiac arrest due to increase in tissue oxygenation in the brain.

8928-42, Session 8

Optical stimulation of the hearing and deaf cochlea under thermal and stress confinement condition

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Optical stimulation of the cochlea, is discussed as a possible alternative to conventional cochlear implants with the hypothetical improvement of dynamic range, frequency resolution.

We investigated optical stimulation of the hearing and deafened guinea pig cochlea in vivo. As laser source we used a laser system tunable to different wavelengths (420nm–2150nm) delivering nanosecond pulses with 6µJ pulse energy via multimode optical fiber. Additionally we utilized another laser at a wavelength around 1850nm with a variable pulse duration from 10µs to 20ms. The optical fiber was positioned in the cochleostomy of the basal turn. Cochlear responses were measured using registration of compound action potentials (CAPs) and recording the excitation pattern in inferior colliculus (IC). Deafening was performed via intracochlear neomycin injection and monitored via frequency specific cochlea responses (CAP and/or IC). Animals were called "deaf" if there were no responses (CAP/IC) measured at 90dB SPL in the frequency range of 500Hz to 32kHz.

In stress confinement the cochlear responses of the normal hearing cochleae showed a correlation to the absorption coefficient of hemoglobin and water, depending on the stimulation wavelength. At one wavelength in NIR the pulse duration range was extended from nanoseconds to milliseconds by implementing both lasers. This allows observing stress and thermal confinement. With intact hair cells cochlea responses similar to acoustic stimulation were measured, while the deafened cochleae did not show any response in any case.

We conclude that in both paradigms the stimulation effect is of optoacoustic nature and relates functional hair cells.

8928-43, Session 8

Localization of changes in optical backscattering during seizure progression in vivo with optical coherence tomography

Melissa M. Eberle, Carissa L. R. Rodriguez, Jenny I. Szu, Univ. of California, Riverside (United States); Yan Wang, Harvard Medical School (United States); Mike S. Hsu, Devin K. Binder, Univ. of California, Riverside (United States) and Umbrella Neurotechnologies Inc. (United States); B. Hyle Park, Univ. of California, Riverside (United States)

Epilepsy is the occurrence of periodic and unpredictable seizures. Current electrical techniques such as electroencephalography (EEG) and electrocorticography (ECoG), or fluorescent techniques such as GCaMP, lack the spatiotemporal resolution and, in particular, the depth discrimination necessary for studying the spatial progression of seizures through the brain. Optical coherence tomography (OCT) is a label-free, high resolution, minimally-invasive imaging tool, which can produce millimeter depth resolved cross-sectional images. We previously identified changes in the backscattered intensity of infrared light, which occurred during the development of induced seizures in vivo in mice. In a large region of interest, we observed significant decreases in the OCT intensity from cerebral cortex tissue preceding and during generalized tonic-clonic seizures induced with pentylenetetrazol (PTZ). In this current study, we leveraged the full spatiotemporal resolution of OCT by studying the temporal evolution of localized changes in backscattered intensity in three dimensions. We conducted both global (induced with PTZ) and focal (induced with 4-aminopyridine (4-AP)) seizure models and analyzed the differences in seizure propagation in the time-resolved 3D functional maps. To compare the electrical and optical temporal evolution, we included implanted electrodes for EEG analysis in our 4-AP model. We also utilized Doppler OCT in both seizure models to discern the changes in vasculature from the observed changes in intensity during seizure progression. The results from this study demonstrate the potential utility of OCT as a label-free, minimally-invasive tool to study brain function with high spatiotemporal resolution.

8928-103, Session 8

Infrared neural stimulation (INS) inhibits electrically evoked neural responses in the deaf white cat

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No Abstract Available

8928-104, Session 8

Target structures in the cochlea for infrared neural stimulation (INS)

Hunter Young, Northwestern Univ. (United States); Xiaodong Tan, Northwestern State Univ. (United States); Claus-Peter Richter, Northwestern Univ. (United States)

No Abstract Available

8928-44, Session 9

Multimodal optoacoustic and two photon microscopy for simultaneous imaging of fluorescence and absorption contrasts *(Invited Paper)*

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Two photon laser scanning microscopy is a common and powerful imaging modality for structural and functional imaging with sub-cellular resolution deep inside a scattering biological tissues. However, this method only images of fluorescence contrast, while other contrasts are at most implied by omission (absence of fluorescence).

Optoacoustic imaging is a powerful technique for non fluorescent absorption contrast. It employs short laser pulses to acquire and reconstruct an acoustic image of the optical absorption contrast, and has a much deeper penetration depth than pure optical imaging.

In a recent publication our group has demonstrated the feasibility of photo-absorber induced neural–thermal stimulation. In this work holographic laser light patterns are projected onto exogenous photo-absorbing particles dispersed in a two-dimensional rat cortical cell culture, thus activating the adjacent cells. The method can be easily generalized for three-dimensional cortical cultures, provided it is possible to determine the location of both the neural cells and the absorbers within the scattering culture in order to construct the required 3D pattern.

Here we describe a low frequency optoacoustic signal from absorbing particles, heated by a high repetition rate femtosecond infrared laser that is standardly used for two photon imaging. This contrast can be imaged simultaneously with standard two photon fluorescence contrast to create a multimodal high resolution 3D modality that enables the implementation of 3d photo-absorber induced neural–thermal stimulation in a scattering medium.

8928-45, Session 9

Development of optical neuroimaging to detect drug-induced brain functional changes in vivo *(Invited Paper)*

Congwu Du, Yingtian Pan, State Unversity of New York at Stony Brook (United States)

Deficits in prefrontal function play a crucial role in compulsive cocaine use, which is a hallmark of addiction. Dysfunction of the prefrontal cortex might result from effects of cocaine on neurons as well as from disruption of cerebral blood vessels. However, the mechanisms underlying cocaine's neurotoxic effects are not fully understood, partially due to technical limitations of current imaging techniques (e.g., PET, fMRI) to differentiate vascular from neuronal effects at sufficiently high temporal and spatial resolutions. We have recently developed a multimodal imaging platform which can simultaneously characterize the changes in cerebrovascular hemodynamics, hemoglobin oxygenation

and intracellular calcium fluorescence for monitoring the effects of cocaine on the brain. Such a multimodality imaging technique (OFI) provides several uniquely important merits, including: 1) a large field-of-view, 2) high spatiotemporal resolutions, 3) quantitative 3D imaging of the cerebral blood flow (CBF) networks, 4) label-free imaging of hemodynamic changes, 5) separation of vascular compartments (e.g., arterial and venous vessels) and monitoring of cortical brain metabolic changes, 6) discrimination of cellular (neuronal) from vascular responses. These imaging features have been further advanced in combination with microprobes to form micro-OFI that allows quantification of drug effects on subcortical brain. In addition, our ultrahigh-resolution ODT (μODT) enables 3D microangiography and quantitative imaging of capillary CBF networks. These optical strategies have been used to investigate the effects of cocaine on brain physiology to facilitate the studies of brain functional changes induced by addictive substance to provide new insights into neurobiological effects of the drug on the brain.

8928-46, Session 9

Detection of cerebral edema in vivo using optical coherence tomography

Carissa L. R. Rodriguez, Jenny I. Szu, Melissa M. Eberle, Univ. of California, Riverside (United States); Yan Wang, Harvard Medical School (United States); Mike S. Hsu, Devin K. Binder, Univ. of California, Riverside (United States) and Umbrella Neurotechnologies Inc. (United States); B. Hyle Park, Univ. of California, Riverside (United States)

Cerebral edema, an increase in brain water content, forms in response to a variety of conditions and leads further clinical complications. Therefore, early detection and timely treatment are vital for improving neurological outcome. In this study we examine the optical changes caused by cerebral edema in vivo with optical coherence tomography (OCT), an optical imaging modality that produces cross-sectional images of biological tissue with micrometer resolution.

A spectral-domain OCT setup centered at 1300 nm was used to image a water intoxication mouse model of cerebral edema. The mouse was anesthetized and prepared with a thin skull cortical window which allowed for an area of 4 x 4 x 2 mm to be imaged. Twenty minutes of baseline recordings were collected followed by an intraperitoneal injection of water to induce cerebral edema. OCT acquisition continued until the animal expired.

Analysis of average OCT intensity from the cerebral edema mouse model showed a continuous decrease over time as cerebral edema progressed. The intensity decreased by approximately 15% by the end of the experiment, one hour after injection. This trend was observed in smaller regions of the brain as well as globally. Furthermore the cerebral blood flow, detected using Doppler OCT, slowed during severe edema. The results showcase the ability of OCT to detect cerebral edema from structural as well as blood flow changes and highlight the potential for OCT to be used clinically for early edema detection.

8928-47, Session 9

Imaging of rat brain using short graded-index multimode fiber

Manabu Sato, Takahiro Kanno, Syoutarou Ishihara, Hiroshi Suto, Toshihiro Takahashi, Reiko Kurotani, Hiroyuki Abe, Yamagata Univ. (Japan); Izumi Nishidate, Tokyo Univ. of Agriculture and Technology (Japan)

Clinically it is important to image structures of brain at the deeper area with low invasions, because the pathological information is not obtained enough from the white matter. Preliminarily we have measured transmission images of rat brain using the short graded index multimode fiber(SMMF) with diameter of 140 ?m and length of 5 mm. SMMF(core diameter 100 ?m, NA 0.29) was cut using a fiber cleaver and was fixed in a jig. Fiber lengths inside and outside jig were 3 mm and 2 mm, respectively. The jig was attached at the 20x objective lens. The conventional optical microscope was used to measure images. In basic characteristics, it was confirmed that the imaging conditions almost corresponded to calculations with ABCD matrix and the spatial resolution was evaluated at about 4.4 ?m by measuring the test pattern. After euthanasia the rat brain at parietal was excised with thickness around 1.5mm and was set on the slide glass. The tissue was illuminated through the slide glass by the bundle fiber with halogen lamp. The tip of SMMF was inserted into the tissue by lifting the sample stage. The transmission image at each depth from 0.1mm to 1.53mm was measured. Below 1.2mm granular structures to show fibrous tissues were recognized. Similar images were measured in a few other sites. Total measurement time was within 2 hours. The feasibilities to image the depth of 5 mm with SMMF were shown. The optics with SMMF and GRIN lens is under investigations.

8928-48, Session 10

Resting-state functional connectivity of cortical networks based on spontaneous optical neural and hemodynamic signals *(Invited Paper)*

Pengcheng Li, Bing Li, Rui Liu, Qin Huang, Jinling Lu, Britton Chance Ctr. for Biomedical Photonics (China)

Resting-state functional connectivity (RSFC) of spontaneous hemodynamic fluctuations is widely used to investigate large-scale functional brain networks. Spontaneous electrophysiological signals such as electroencephalograph (EEG), local field potential (LFP), were investigated to understand the neural basis of RSFC in previous studies. However, because of the limited spatial resolution of the recorded electrophysiological signals, there is still a lack of the direct comparison between the spatial pattern of functional connectivity based on high resolution neural activity signal and that based on hemodynamic signals to understand the underlying neural mechanisms of RSFC. Moreover, it is necessary to investigate whether the RSFC based on low-frequency fluctuations of hemodynamic signal can remain to reflect the functional brain networks during those conditions that neurovascular uncoupling may occur. Here we reported the RSFC of neural activity measured by optical imaging of voltage-sensitive dyes, which could provide a high spatial resolution, within the motor, somatosensory and visual cortexes of mice. Although it is found that RSFC of neural activity of slow cortical potentials (0.1-4 Hz) had a similar spatial pattern of correlation as that of spontaneous hemodynamic signals, the RSFC of neural activity between bilateral cortexes was significant asymmetry, which can also be observed in the functional activation pattern evoked by the sensory stimulations. Furthermore, the coupling between RSFC of slow cortical potentials and that of hemodynamic signals was attenuated by increasing anesthetic levels, and RSFC of hemodynamic signals became discrete in the sensorimotor cortex during deep anesthesia. Our results disclose the RSFC of neural activity with a high spatial resolution and suggest that spontaneous hemodynamic signals are coupled with slow cortical potentials at resting state. The comparison between RSFC of neural activity and that of hemodynamic signals indicates that the slow cortical potentials may have a better localization of RSFC networks than spontaneous hemodynamic signals, especially under deep anesthesia.

8928-49, Session 10

High speed imaging of mouse brain cortical spontaneous activity provides insight into regional connectivity *(Invited Paper)*

Timothy H Murphy, Majid Mohajerani, Allen Chan, Matthieu Vanni, Yiecheng Xie, University of British Columbia (Canada)

Using millisecond-timescale voltage-sensitive dye imaging in lightly anesthetized or awake adult mice, we show that a palette of sensory-evoked and hemisphere-wide activity motifs are represented in spontaneous activity. These motifs can reflect multiple modes of sensory processing, including vision, audition and touch. We found similar cortical networks with direct cortical activation using channelrhodopsin-2. Regional analysis of activity spread indicated modality-specific sources, such as primary sensory areas, a common posterior-medial cortical sink where sensory activity was extinguished within the parietal association area and a secondary anterior medial sink within the cingulate and secondary motor cortices for visual stimuli. Correlation analysis between functional circuits and intracortical axonal projections indicated a common framework corresponding to regional long-range monosynaptic connections. Similar maps were observed with other indicators of neuronal activity such as intracellular calcium and measures of neurotransmitter release.

8928-50, Session 10

Backscattered OCT intensity changes during seizure activity

Md. Rezuanul Haque, Michael C. Oliveira, M. Shahidul Islam, Gregory N. Filatov, Mike S. Hsu, Devin K. Binder, Maxim Bazhenov, B. Hyle Park, Univ. of California, Riverside (United States)

Epilepsy is a neurological disorder characterized by periodic and unpredictable occurrence of seizures. Using noncontact NIR imaging and fiber-optic probes, previous studies identified optical changes during seizures in mice brain in vivo, although the precise temporal and spatial correlation between optical changes and seizures was not examined due to limitations in spatial resolution of both optical and electrophysiological acquisition systems. Optical coherence tomography (OCT) is a minimally-invasive technology capable of rapid two- and three-dimensional imaging of 2-3mm of subsurface tissue structure with micrometer resolution. In this study, OCT was used to identify intensity changes of the backscattered light and their relation to simultaneously acquired multi-electrode array (MEA) measurements was analyzed. The acute hippocampus region of the mice brain slice was simultaneously monitored using volumetric OCT imaging and MEA recordings before, during and after seizure activities. En face images were calculated by integrating the depth-wise intensity (z-direction) of the 3D OCT volumetric data during post processing. Optical and electrical activity changes were color-coded and overlaid on integrated en face image to visualize spatial-temporal correlation. The results showed a strong correlation between optical changes and electrical activity. These results also suggest that OCT can be used as a potential tool for epilepsy diagnostics and to gain a better understanding of the mechanism of seizures.

8928-51, Session 10

Fiber bundle system for deep brain imaging

Ling Fu, Britton Chance Ctr. for Biomedical Photonics (China)

Neural network activity is accompanied with dynamic transients of calcium ion concentration, which can be detected by optical method with calcium sensitive dyes. For deep brain calcium signal detection in a freely moving animal, optical fiber-based approaches are optimal and have been used in many brain function researches. Among them, the single multi-mode fiber (MMF) approach is most commonly used, which can collect the calcium signal of a clusters of neurons without spatial resolution. More fibers can be used to construct a multi-channel system, but it's complicated and will cause more injury. We developed a fiber bundle system for deep brain calcium imaging. The fiber bundle which is 750 μm in diameter with 30000 fibers, can provide about 3.5 μm lateral resolution at the distal end of the bundle without causing much more injury than the usually used MMF. Because the flexibility of the fiber bundle is not as good as the MMF, it may be winded to broken when used in freely moving animal. We used a customized fiber optic rotary joint to fix the proximal end of the bundle, so that the bundle will rotate along with the moving animal. The resulting rotary of image can be calibrated by subsequent data processing and the image sequences can provide calcium transients in time at any position of the imaging field as what the single fiber approach work for. This imaging system will be useful to provide time sequence and spatial distribution of calcium signal simultaneously. With an intensity tunable light source, it can also be used in optogenetics for optical stimulation and recording.

8928-69, Session PMon

Cerebral hemodynamics in patients with obstructive sleep apnea syndrome monitored with near-infrared spectroscopy (NIRS) during positive airways pressure (CPAP) therapy: a pilot study

Zhongxing Zhang, Univ. of Zürich (Switzerland); Maja Schneider, Ursula Fritschi, Isabella Lehner, Ming Qi, Center for Sleep Medicine and Sleep Research, Clinic Barmelweid (Switzerland); Ramin Khatami, Klinik Barmelweid (Switzerland)

In obstructive sleep apnea syndrome (OSA) the periodic reduction or cessation of breathing due to narrowing or occlusion of the upper airway during sleep leads to daytime symptoms and increased cardiovascular risk, including stroke. The higher risk of stroke is related to the impairment in cerebral vascular autoregulation. Continuous positive airways pressure (CPAP) therapy at night is the most effective treatment for OSA. However, there is no suitable bedside monitoring method evaluating the treatment efficacy of CPAP therapy, especially to monitor the recovery of cerebral hemodynamics. NIRS is ideally suited for non-invasive monitoring the cerebral hemodynamics during sleep. Several NIRS studies characterized the hemodynamics during sleep in patients with sleep disorders including OSA. In this study, we will first time assess dynamic changes of cerebral hemodynamics during CPAP therapy in 3 patients with OSA using NIRS. We found periodic oscillations in HbO2, HHb, tissue oxygenation index (TOI) and blood volume associated with periodic apnea events without CPAP in all OSA patients. These oscillations were gradually attenuated and finally eliminated with the stepwise increments of CPAP pressures. The oscillations were totally eliminated in blood volume earlier than in other hemodynamic parameters. These results suggested that 1) the cerebral hemodynamic oscillations induced by OSA events can effectively be attenuated by CPAP therapy, and 2) blood flow and blood volume recovered first during CPAP therapy, followed by the recovery of oxygen consumption. NIRS is a useful tool to evaluate the efficacy of CPAP therapy in patients with OSA bedside and in real time.

8928-70, Session PMon

Pilot study to compare the cerebral hemodynamics between patients with obstructive sleep apnea syndrome (OSA) and periodic limb movement syndrome (PLMS) during nocturnal sleep with near-infrared spectroscopy (NIRS)

Zhongxing Zhang, Univ. of Zürich (Switzerland); Maja Schneider, Ramin Khatami, Clinic Barmelweid (Switzerland)

Obstructive sleep apnea syndrome (OSA) and periodic limb movement in sleep syndrome (PLMS) are two common sleep disorders. Previous studies showed that OSA and PLMS share common features, such as increased cardio-vascular risk, both apnea events and limb movements occur periodically, they are usually associated with cortical arousals, and both of them can induce declines in peripheral oxygen saturation measured with pulse oximetry. However, the question whether apnea events and limb movements also show similar characteristics in cerebral hemodynamic and oxygenation has never been addressed. In this pilot study, we will first time compare the cerebral hemodynamic changes induced by apnea events and limb movements in patients with OSA (n=5) and PLMS (n=4) with NIRS. In patients with OSA, we found periodic oscillations in HbO2, HHb, and blood volume induced by apnea events, HbO2 and HHb showed reverse changing trends, and there were phase delay between the oscillations in HbO2 and blood volume. By contrast, the periodic oscillations linked to limb movements were only found in HbO2 and blood volume in patients with PLMS, and there were no phase shift between HbO2 and blood volume oscillations. These findings of different cerebral hemodynamics patterns between apnea events and limb movements may indicate different regulations of autonomic nervous system between these two sleep disorders.

8928-71, Session PMon

Fluorescence holography with enhanced interference extent and improved signal-to-noise

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We present a system that can realize near focal plane detection with high extent of interference benefit from its small optical path difference (OPD). This is achieved by set the focal length of the lens for reference wave close to that for object wave. The signal-to-noise ratio (SNR) is improved by about three times compared with the conventional technique, and a signal 4.6 times weaker can be detected.

8928-72, Session PMon

The effect of RGB monochromatic and polychromatic LED lighting on growth performance, behavior, and development of broilers

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In modern poultry production, artificial illumination is one of the most important scientific and technological subject of interest owing to its effect on the regulation and control of behavior and health of most animals. In particular, light schedule, intensity of illumination, and wavelength of light has proven to be major factors influencing growth and well-being of broilers. The wavelength sensitivity curve of avian eye, like the human eye, covers the visible (380-720 nm) spectral region. However, broilers can detect a broader spectral region than humans, especially in the UV presenting peak sensitivity in four distinct regions 375, 485, 550, and 645 nm. In addition to the eyes, birds are equipped with active extra-retinal photo-sensors. These photo-sensors residing in the brain can collect light energy through the skull and tissues, which are responsible for transduction in photo stimulation. Therefore, the exploitation of colored lighting in modern broiler husbandry is increasing and attracting much attention recently. Red light has proven to reduce aggression and cannibalism in free range layers. However, red light illumination causes decrease in broilers productivity due to decrease in testosterone levels. On the other hand, UV, blue, and green light sources effectively stimulates testosterone secretion, and myofiber growth both leading to increasing body growth, and better productivity. Light-emitting-diode (LED) based illuminants are regarded as the next generation lighting technology. The LED-based sources incorporate valuable properties including controllable wavelength and power, exhibit low power consumption, high electrical energy to light conversion efficiency, long-life, and low-cost and easy maintenance. In addition, they possess the capability of producing RGB monochromatic and/or multicolor light combination with controlled intensity, and wavelength. Growth performance, behavior, and development of broilers reared under red, green, and blue monochromatic and/or multicolor LED-based illuminants is investigated. The lighting treatments were performed on a 24h lighting basis during six weeks. Monochromatic red(630 nm), green(520 nm), and blue(460 nm), and simultaneous blue-green, and white-light housing illumination was employed. Bodyweight, food consumption, and behavior were monitored and compared amongst light treatments. Results showed that broilers under blue and/or green monochromatic illumination exhibited more than 6% increase in bodyweight when compared to those under red or white-light. The highest and lowest feed conversion was observed in broilers reared under blue and red illumination, respectively, when compared to white-light lighting

8928-73, Session PMon

Fluorescence micro-optical sectioning tomography for brain circuits visualization

Xiaoli Qi, Xiaohua Lv, Anan Li, Hui Gong, Qingming Luo, Shaoqun Zeng, Huazhong Univ. of Science and Technology (China)

Mapping neuronal circuits is essential for understanding the brain function, and fluorescence microscopic imaging plays crucial roles in drawing single-fiber-resolution neuronal connectivity map. Recently reported Micro-Optical Sectioning Tomography (MOST) system is potential to draw the neuronal circuits of large brain volume with submicron resolution by combining fine mechanic sectioning with simultaneous optical imaging.

The major problem that makes fluorescence MOST (a knife-edge imaging system) impractical is, the image loss in the tear burr induced when sectioning lead to interruptions in continuous fiber tracing and the neuronal circuits. The percentage of that area compared to the whole area is very small. However, any interruption in neuronal circuits might result in failure of the whole circuits. A similar example in the field of electronic engineering is, a single failure welding in the electronic circuits of the cell phone would result in dysfunction of the whole phone. So here in this paper we pay special efforts to recover the information in the tear burr area. We introduced confocal detection to recover the interruptions of the nerve fiber. We demonstrated that with a 50-?m–width confocal slit, the signal-to-background ratio is increased 16~49 fold than that without the slit, which effectively improves the detectability of the signal in the interruptions and enables continuous tracing of the neuronal networks.

8928-74, Session PMon

Enhancing the precision of neurite tracing for brain circuits via directional filtering

Jing Li, Tingwei Quan, Shiwei Li, Hui Gong, Qingming Luo, Shaoqun Zeng, Huazhong Univ. of Science and Technology (China)

Digital reconstruction of neurons is indispensable for quantifying neuronal morphology and extracting the structure of neuronal circuits. Neurite tracing, as the most important part of digital reconstruction of neurons, has been studied widely. Recent progress in molecular labeling and microscopy imaging system makes high-throughput acquisition of neuronal datasets become a reality and provides a base for reconstructing the big brain circuit. However, due to the fact that the neurons in the brain circuit were distributed in different regions of brain, these neuron's signals vary sharply, even some signal can't be distinguished from the background. In this case, Neurite tracing experiences the huge difficulties. Here, by using the multi-windows for selecting seeds, ray-burst sampling for extracting local signals and principal component analysis for computing the principal directions, we developed a novel directional filtering method. Compared to other directional filtering methods, our method significantly increases the intensity of the weak neuronal signals, meantime, efficiently constrains the enlargement of radius of neurites. The results show that our directional filtering is useful for neurite tracing methods like Neuron Studio, and assure that the Neuron Studio can analyze the weak signals of neurons.

8928-75, Session PMon

Classification of various mental task combinations for functional NIRS-based brain-computer interface

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The goal of this study was to investigate the most suitable combinations of mental tasks for the development of practical near-infrared spectroscopy (NIRS)-based brain-computer interface (BCI) systems. To this end, we recorded concentration changes of oxygenated [oxy-Hb] and deoxygenated [deoxy-Hb] hemoglobins while seven participants were performing eight different mental tasks; left hand motor imagery, right hand motor imagery, foot motor imagery, internal singing, mental subtraction, mental multiplication, geometric figure rotation, mental character writing. Four different feature sets were extracted from the recorded NIRS signals ([oxy-Hb], [deoxy-Hb], [total-Hb], and a combination of [oxy-Hb] and [deoxy-Hb]), and classification accuracies were estimated for all possible pairs of the eight mental tasks (8C2 = 28). Linear discriminant analysis with a 10x10 cross-validation method was used for evaluating classification accuracy. As a result, three mental tasks, right hand motor imagery, mental multiplication, and geometric figure rotation, were mostly selected in mental task combinations showing accuracy high enough for practical communication (> 70 %). In particular, a combination of right hand motor imagery and geometric figure rotation task only showed high classification accuracy over 70 % on average, when using the feature set of the combination of [oxy-Hb] and [deoxy-Hb]. From the results, it was confirmed that the combination of right hand motor imagery and geometric figure rotation task can be a promising candidate task for a practical NIRS-based BCI system.

8928-76, Session PMon

Range-dependent synchronized bursts in dissociated neuronal networks

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Spatial-temporal configurations of electrophysiological activities are believed to contribute to neural information processing and synaptic formation. Although synchronized bursts in the hippocampus are correlated with memory consolidation and brain diseases, the mechanism underlying these activities of neural networks still remains poorly understood. With multi-electrode arrays, spontaneous synchronized bursts were found in high-density hippocampal networks after 20 days in vitro. The initiating sites of these bursts, which estimated from relative delays of onsets of activities between electrodes, distributed randomly from individual bursts. Our statistical data confirms that the spatial layout of such initiating sites is stable. To determine the propagation characteristics of these bursts, a femtosecond laser was utilized for cutting the network in different size around the initiate site. After cutting, the spreading and connectivity of the synchronized activities were disturbed while synchronized bursts rate decreased. The synchronized activities disappeared if network diameter was reduced to < 200 μm. Moreover, statistical results indicated the synchronous degree of bursting activities was closely related to the range of active neurons in the network. In conclusion, by combing multi-electrode array and femtosecond laser, we studied the temporal evolution and spatial distribution of synchronized burst in the self-organized hippocampal networks in vitro, which showed range-dependent characteristics.

8928-77, Session PMon

Quantitative assessment of brain tissue oxygenation in porcine models of cardiac arrest and cardiopulmonary

resuscitation using broadband near-infrared spectroscopy

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Near-infrared spectroscopy (NIRS) is a powerful tool to measure real-time tissue oxygenation in the brain. We used a broad-band, continuous-wave hyper-spectral approach to measure tissue oxygenation in the brain of piglets under the conditions of cardiac arrest, cardiopulmonary resuscitation (CPR), and defibrillation. The purpose of this research was to find a correlation between mortality due to cardiac arrest and inadequacy of the tissue perfusion during attempts at resuscitation. Using this technique we measured the changes in concentrations of oxy-hemoglobin and deoxy-hemogolobin to quantify the tissue oxygenation in the brain. We also extracted cytochrome c oxidase changes under the same conditions to determine increase or decrease in cerebral oxygen delivery. In this paper we proved that applying CPR, oxy-hemoglobin concentration and brain tissue oxygenation in the brain increase while deoxy-hemogolobin concentration decreases which was not possible using other measurement techniques. We concluded that improving CPR methodology could increase the patient survival chance after a cardiac arrest due to increase in tissue oxygenation in the brain.

8928-78, Session PMon

Depth resolved optical detection of nerve activity in Limulus nerve and murine brain slice using common-path OCT

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This study is aimed to develop an optical tool to image the changes in nerve structures that correspond with neural activity. The majority of current technologies use either different varieties of electrodes or exogenous contrast agents for neural recording, with both arguably being invasive to some extent. Nerves undergo small rapid transient thickness change during action potential propagation and this sub-nanometer thickness change has temporal correlation with action potential. Our developed common path pr-OCT system is currently capable of detecting thickness changes as small as 500 picometers with a temporal resolution of 10?s. Recent optical measurements from Limulus optic nerve and murine brain slice have shown evidence of concurrent optical changes associated with neural activity. Averaging of 8-10 impulses improves the SNR of detected optical signal. However, most of the results demonstrated here are single shot detection. Since OCT collects data from every depth points within a single A-line simultaneously, we have examined the changes in phase at every depth location. Results demonstrate that these transient changes are present at different depths and this allows representing activity as a map of thickness changes. A custom-built cold block system has been used for switchable control of activation and deactivation of action potential propagation through the Limulus nerve. Optical recording has been compared with simultaneous electrical recording at every stages of cold block operation: activated nerve before deactivation, nerve after deactivation and nerve after reactivation.

8928-79, Session PMon

Optical monitoring of shock wave-induced spreading depolarization and concomitant hypoxia in rat brain

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Blast-induced traumatic brain injury is a growing concern but its underlying pathophysiology and mechanism are still unknown. Thus, study using animal model is needed. We have been proposing the use of a laser-induced shock wave (LISW), whose energy is highly controllable and reproducible, to mimic blast-related injury. We previously observed occurrence of spreading depolarization (SD) and prolonged hypoxia in the rat brain exposed to an LISW. However, relationship between these two events is unclear. In this study, we investigated spatiotemporal characteristics of hypoxia and SD to examine their correlation. Furthermore, we quantified tissue oxygen saturation in the hypoxic phase, which is associated with possible neuronal cell death, based on inverse Monte Carlo simulation. First, we simultaneously performed CCD-based imaging of NIR light scattering and measurement of DC potential for the brain. We observed that propagation of light scattering waves temporally coincided with the DC potential negative shift, indicating that SD caused light scattering change. Secondly, we applied an LISW onto the left frontal cortex and measured diffuse reflectance at three different points on the parietal bone with an optical fiber pair. Hypoxia first started at the nearest position to the LISW source and its onset was delayed at the farther positions; speed of expansion of hypoxia was similar to that of the propagation speed of scattering wave (SD). Simulation showed that oxygen saturation was decreased by ~40%. These results indicate strong correlation between SD and hypoxia, and oxygen saturation was low enough to induce neuronal cell death.

8928-80, Session PMon

Prefrontal hemodynamic responses related with consumer preferences using fNIRS

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In the neuromarketing field, it is desirable to determine and predict the consumer preference based on scientific evidences such as brain activities. Previously, various neuroimaging techniques such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) have been tested to measure the preference. In this study, we utilized a functional Near-Infrared Spectroscopy (fNIRS) technique for the same objective which has the advantage of cost-effectiveness and convenience. A total of 16 healthy, right-handed participants have volunteered for this study. Prior to experiment, 10 most preferred and 10 non-preferred pictures among 100 pictures from 5 categories were pre-selected from the individual survey. During the experiment, participants were instructed to watch the screen that displays 10 preferred or 10 non-preferred image sets for 50 seconds, sequentially. Hemodynamic changes were measured with a wireless fNIRS system attached on the pre-frontal area. The fNIRS system consists of 3 near-infrared light sources and 8 detectors, 12 channels with a sampling rate of 14 Hz. The wavelength of light sources are 780 and 850 nm. We analyzed the amplitudes of the hemodynamic signal changes and also the spatial characteristics for various source-detector channels. The results indicate that statistically significant changes of oxyhemoglobin concentrations are observed in the left dorsolateral prefrontal cortex (dlPFC) area. The hemodynamic responses were also increased in the right ventromedial prefrontal cortex (vmPFC) area. This study suggests that personal preference may be well investigated by monitoring frontal hemodynamic activities using fNIRS.

8928-52, Session 11

Digital mouse brain atlas with high-resolution images *(Invited Paper)*

Hongwei Dong, Univ. of California (United States)

No Abstract Available

8928-53, Session 11

Visible brainwide networks at single-neuron resolution *(Invited Paper)*

Qingming Luo, Wuhan National Laboratory for Optoelectronics-Huazhong University of Science and Technology (China)

A Micro-Optical Sectioning Tomography (MOST) system has been developed for mapping brainwide networks. We will clarify the unique features of the MOST in comparison to other techniques and present a whole-brain approach to systematically obtaining continueous neuronal pathways in a fluorescent protein transgenic mouse at a one-micron voxel resolution.

8928-54, Session 11

Simultaneous visualization of cells and capillaries in an entire mouse brain with one voxel resolution

Jingpeng Wu, Hui Gong, Huazhong Univ. of Science and Technology (China)

Systematic cellular and vascular configurations are fundamental for understanding brain anatomy and metabolism. However, it remains a great challenge to acquire, visualize and quantitative analyze 3D cellular and vascular configurations at the level of individual cells and capillaries in the entire brain. In this study, we introduced a modified Nissl staining optimized for the whole mouse brain to contrast cell body and vascular wall to brain tissue. To estimate sample shrinkage, we scanned and compared the volume of the mouse brain with micro computed tomography. Then, the intact resin-embedded mouse brain was sectioned and imaged uninterruptedly for approximated 170 hours using micro-optical sectioning tomography system with a z stack thickness of 1 um. The voxel size of the completed dataset was 0.35 x 0.4 x 1.0 um3. Based on these datasets, an automatic image-processing pipeline has been developed for analyze the cellular and vascular configurations, including cell detection, vessel tracing and quantitative analyzing tools. Based on this study, our protocol is believed to gain new insights into the morphology, localization and interconnectivity of cells and capillaries throughout the whole brain at a real anatomy architecture resolution.

8928-55, Session 11

Visualization brain circuits using two-photon fluorescence micro-optical sectioning tomography

Ting Zheng, Zhongqing Yang, Anan Li, Xiaohua Lv, Qingming Luo, Hui Gong, Shaoqun Zeng, Huazhong Univ. of Science and Technology (China)

Neural circuits are fundamental for brain functions. However, obtaining long range continuous projections of neurons in the entire brain is still challenging. Here a two-photon fluorescence micro-optical sectioning tomography (2p-fMOST) method is developed for high-throughput, high resolution visualization of the brain circuits. It combine two photon imaging and plastic embedding technology, because the sample was embedded in a plastic embedding manner, thus a high sampling accuracy of submicron could be obtained. Moreover, the focal plane was set to below the surface of the sample for the features of two photon imaging, together with the method of mechanic sectioning after imaging, the cutting requirements was significantly decreased and the integrity of data was maintained.

Based on this novel method, we design and set up a 2p-fMOST system, and acquired a three-dimensional data set of a Thyl-enhanced green fluorescent protein transgenic mouse brain with a resolution of 0.45 ?m in lateral and 1.68?m in axial. After 215 h, we generated this entire brain database with a voxel size of 0.5 ? 0.5 ? 2 ?m3 by 6330 optical sections. In this data set, spines and axons could be easily resolved. Besides, we traced several neural long-distance projections distributed in different brain regions based on this data set because of its high spatial resolution, high voxel resolution and high integrity. The test shows that 2p-fMOST system is a novel tool that can acquire neural functional connection data set in the whole brain with high precision, high speed and high integrity.

8928-56, Session 11

Reconstruction of dense neuronal fibers in three-dimensional fluorescence image stacks

Tingwei Quan, Hang Zhou, Hui Gong, Qingming Luo, Shaoqun Zeng, Huazhong Univ. of Science and Technology (China)

Digital reconstruction of neurons usually can be regarded as using the suitable computing method to quantitatively describe the neuronal morphology from 3D microscope image stacks. This is the key to mapping neuronal circuits [1], identifying the types of neurons [2] and inferring the neuronal firing patterns [3]. Recent progresses in imaging techniques [4-7] can image the whole brain of a mouse at submicron-resolution or micron-resolution, which provides a strong tool for high-throughput acquisition of the image stacks of neurons. However, from these image stacks, digital reconstruction of neurons with high-density spatial distribution still keeps a challenge [8,9], due to the fact that few of tracing methods involved in high-density dataset analysis. Here, by using constrained principal curves [10] and the estimated patterns of neurite connection, we have developed to a novel method for neurite tracing. Our method can efficiently identify bifurcations or crossings in neurite tracing. Our method also can eliminate the interference of other neurons and precisely extract the center-lines of single neuronal morphology from the high-density datasets. When compared to the typical tracing method like open-snake method [11], our tracing method has about 10x faster tracing speed, and significantly boosts the precision in identifying each neuron and its own connections from high density datasets. Combined with our previous works achieving the precise localization and segmentation of neuronal somas [12,13], the proposed neurite tracing method can be easily used for reconstruction of dense neuronal fibers. I believe that our method can provide an important tool in quantifying neuronal morphology, and researching some structural properties of the neuronal population.

8928-57, Session 12

In vivo imaging of neural reactive plasticity after laser axotomy in cerebellar cortex *(Invited Paper)*

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Multi-photon imaging provides valuable insights into the continuous reshaping of neuronal connectivity in live brain. We previously showed that single neuron or even single spine ablation can be achieved by laser-mediated dissection. Furthermore, single axonal branches can be dissected avoiding collateral damage to the adjacent dendrite and the formation of a persistent glial scar. Here, we describe the procedure to address the structural plasticity of cerebellar climbing fibers by combining two-photon in vivo imaging with laser axotomy in a mouse model. This method is a powerful tool to study the basic mechanisms of axonal rewiring after single branch axotomy in vivo. In fact, despite the denervated area being very small, the injured axons consistently reshape the connectivity with surrounding neurons, as indicated by the increase in the turnover of synaptic boutons. In addition, time-lapse imaging reveals the sprouting of new branches from the injured axon. Newly formed branches with varicosities suggest the possible formation of synaptic contacts. Correlative light and electron microscopy revealed that the sprouted branch contains large numbers of vesicles, with varicosities in the close vicinity of Purkinje dendrites.

8928-58, Session 12

In vivo voltage-sensitive dye optical imaging of the brain subcortical structures *(Invited Paper)*

Qinggong Tang, Univ. of Maryland, College Park (United States); Vassiliy Tsytsarev, Univ. of Maryland (United States); Chia-Pin Liang, Univ. of Maryland, College Park (United States); Reha Erzurumlu, Univ. of Maryland School of Medicine (United States); Yu Chen, Univ. of Maryland, College Park (United States)

Localization and real-time monitoring of the neural activity evoked by peripheral stimulation are important steps in understanding the functional characteristics of neuronal circuits in the brain. The rodent vibrissae system is an excellent model to investigate the development, organization, function and plasticity of mammalian sensory pathways. Voltage-sensitive dye imaging (VSDi) offers an opportunity to study the activity of neuronal ensembles in vivo with relatively high spatial (up to 20?m) and temporal resolution (up to few milliseconds) which is comparable with electrophysiology. Currently used fast CCD camera-based VSDi can only provide information from the cortical surface. Gradient refractive index (GRIN) lens that are 350–2,000?m in diameter and provide micron-scale resolution have been used in deep brain imaging with minimal injury. In our research, we combined VSDi with GRIN lens to study neural functions in mice vibrissae system. Neural activities in the ventral posteromedial nucleus of the mice thalamus were imaged in vivo during mechanical whisker stimulation. Different diameter GRIN lenses were used to compare the image quality and evaluate the influence of needle to neuron activities. We also compared latency and temporal structure of signals obtained from the cortex and from the thalamus. By localizing responses obtained in reply to different whiskers stimulation, we obtained a functional map of the barreloids for the first time. Localization of the GRIN lens tip has been validated histologically. The proposed method can be easily adapted to study different neuronal circuits, which will provide neuroscientists with new tools to investigate neural circuits.

8928-59, Session 12

Femto-second pulsed laser induced astrocytic calcium wave

Wei Zhou, Yuan Zhao, Britton Chance Ctr. for Biomedical Photonics (China)

Previous studies show astrocyte has bidirectional communications with neurons by forming tripartite synapses. The key technique for this purpose is to induce astrocytic Calcium signaling. However, conventional methods cannot stimulate cells in noncontact and high spatiotemporal precision way. Here, photostimulation of astrocyte with the femtosecond laser was used to study the integration in single astrocyte and astrocyte-to-neuron signaling.

At first, femtosecond laser, which focused on the astrocytic membrane, induced intercellular calcium wave that subsequently spread out from the target cell in a radial pattern and ultimately lead to the activation of the astrocyte network. Additionally, photoporation was suggested to be localized and transient, without compromising cell viability and functions.

To investigate the integration in single astrocyte, the pair stimulation modes were developed on our custom-built random-access scanning two-photon fluorescence microscope by the acousto-optic deflector. Three propagation patterns of Calcium wave in the stimulated cell were observed: delayed, slowed and mixed. The results showed that Ca2+ signaling was inclined to react earlier with fast rising phase and sublinear summation of the amplitude.

To study the astrocyte-neuron interaction, astrocytic activity was induced by photostimulation with the femtosecond laser. Neuronal reaction time is linearly related to the distance from the stimulated cell. Moreover, neuronal synchronous oscillations were observed to be modulated by astrocyte activity. Induction of synchronous oscillations, instantaneous interference of the synchronous circuit, pacemaking effects on the spontaneous high-frequency synchrony in the neural circuit were discovered, respectively.

8928-60, Session 12

Chemical reactivating fluorescent protein molecules enables large scale resin-embedded fluorescence micro-imaging

Hanqing Xiong, Qingming Luo, Hui Gong, Shaoqun Zeng, Huazhong Univ. of Science and Technology (China)

Resin embedding method have been well developed and broadly used as a basic tool on almost all the micro imaging fields, since it was first proposed to ensure ultrathin section specimen preparation for transmission electron microscopy in 1949. The good sectioning property of resin embedded tissue helps to successfully break the barrier that limits microscope to image thick tissues, which has long inspired people to consider using it on fluorescent protein labeled specimen. However, a fatal drawback of the resin embedding method impeded us. it is not compatible with the powerful modern developed fluorescent protein labeling and imaging technique: the resin embedded procedure presents to quench the most often used GFP and its variants, which induced poor fluorescent contrast and even detection fail for well-labeled specimen. This disadvantage impedes its usage on modern molecular biology researches. many efforts has been taken to improve the fluorescence preservation ability of resin embedding method. Since all these methods are based on optimizing the embedding protocol, none of them touch the essence of the fluorescent protein quenching. As a result, the fluorescence intensity in resin embedded tissue remains decreasing, and whether the labeled structure can be preserved and detected remains unknown. Here we report the essence that causes GFP and its variants quenching in resin, and find a way to recover the quenched fluorescent protein, and enable successful fluorescence imaging of large scale resin-embedded mouse brain.

8928-61, Session 13

Neural circuit plasticity in the motor cortex in mouse models of Parkinson's disease *(Invited Paper)*

Tonghui Xu, Huazhong Univ. of Science and Technology (China); Jun Ding, Stanford Univ. School of Medicine (United States)

Parkinson's disease (PD) is a chronic neurodegenerative disorder resulting from the loss of dopamine neurons in the brain. One of the hallmarks of PD is that patients show a decline in motor learning in addition to the widely recognized difficulties in motor execution. However, the mechanism is unknown. To date, studies have been largely focused on corticostriatal projections and their adaptations in PD. It is unclear how loss of dopamine may directly affect the dynamic plasticity of motor cortex, which is crucial in encoding motor learning and plasticity. To determine if and how the plasticity of motor cortex circuits is affected, we use transcranial two-photon microscopy to track the circuit plasticity structurally in vivo in several mouse models of PD (MPTP, Reserpine and 6-OHDA models). We found that the structural plasticity of motor cortex in dopamine deficient mice was significantly influenced. The turnover rate of dendritic spines in layer V pyramidal neurons in vivo increased more than 5% following the loss of dopamine. In addition, the long-term synaptic plasticity in layer V cortical neurons is impaired in vitro. These results point to a novel mechanism that is responsible for the deficiency in motor learning in PD.

8928-62, Session 13

Functional NIRS-based brain-computer interface for the classification of covert yes and no intentions

Chang-Hwan Im, Han-Jeong Hwang, Hanyang Univ. (Korea, Republic of)

Functional near-infrared spectroscopy (fNIRS) has become one of the most promising neuroimaging modalities in recent brain-computer interface (BCI) research. The goal of this study was to decode fNIRS signals to classify participants' covert 'yes' and 'no' intentions for the development of a practical fNIRS-based BCI system. Eight healthy participants took part in this study. They were asked to internally answer to simple seventy 'yes/no' questions (e.g., Are you thirsty?), during which we recorded concentration changes of oxygenated [oxy-Hb] and deoxygenated [deoxy-Hb] hemoglobins using NIRS optodes attached on the participant's scalp. To extract features for classification, common spatial pattern (CSP) was applied to the raw [oxy-Hb] and [deoxy-Hb] data, and then the original data were transformed into new time series with distinct spatial patterns. The variances of the converted [oxy-Hb] and [deoxy-Hb] data were used as features, and the classification accuracy was estimated for each of the [oxy-Hb] and [deoxy-Hb] data. As a result, high classification accuracy over 80 % could be obtained even when only 5 s analysis window was used ([oxy-Hb]: 82.42 %, 84.13 % and 83.39 % for 5 s, 7.5 s and 10 s; [deoxy-Hb]: 81.74 %, 85.52 %, 84.73 % for 5 s, 7.5 s and 10 s), demonstrating that fNIRS could be used to discriminate covert 'yes/no' intentions with high accuracy. It is expected that the proposed paradigm would be used as a binary communication system for those who are in completely locked-in state (CLIS) or minimally conscious state (MCS).

8928-63, Session 13

Detection of action potentials in a bulk neural tissue via intrinsic properties of neurons

Olivier Thouvenin, A. Claude Boccara, Mathias Fink, Institut Langevin (France)

During action potentials, physical properties of neurons membrane change drastically since an important part of its components are charged molecules. Mechanical deformations triggered by neural activity are also expected in the tissue. These changes can be monitored via neurons optical properties. Their detection would be interesting in neuroimaging, since no chemical or genetic manipulation, that becomes increasingly difficult with organism "complexity", would be needed to follow non invasively single neuron activity. Previous studies have demonstrated that optical properties of nerves and giant axons do change during activity, notably birefringence properties and optical path. Nevertheless, at our knowledge, such effects have never been observed in mammalian cortex neurons with cellular resolution in real time. Moreover, the biological origin of these optical changes are still being discussed.

We used an original setup of Ultra-Fast Full-Field Optical Coherence Tomography able to detect nanometric changes in the optical path at 20,000 images/s. To date, we succeeded in measuring a nanometric phase shift in neuron cultures from rat hippocampus. We plan to add a Structured Illumination Microscopy part to the setup able to detect fluorescence increase of chemically marked active neurons to demonstrate the correlation between optical changes and action potentials. We will also perform similar measurements in intact animals. The high acquisition rate will finally allow us to investigate whether shear waves propagate into the medium after optical changes. It would mean that the latter are initiated by mechanical deformations induced by neurons activation, helping us to better understand their origin.

8928-64, Session 13

Optical clearing cranial window for imaging cortical blood flow

Yang Zhang, Jing Wang, Tonghui Xu, Qingming Luo, Dan Zhu, Huazhong Univ. of Science and Technology (China)

Cortical blood flow monitoring with LSCI has provided valuable insight into many aspects of cerebral vascular structural and functional architecture in cortex at high temporal-spatial resolution. In order to overcome the effect of turbid skull above the cortex, the previous investigations were performed by craniotomy. Exposed cortex by removing skull is an available method, but is only suitable for short-term observation. Those developed chronic cranial windows, such as thinned-skull cranial window, open skull glass window et al. offer useful models for long-term observation, but these models are not only complex to perform, but also dissatisfactory.

Here, an innovative optical clearing method was developed to clear the skull within tens of minutes by topical treatment of optical clearing agents, which makes it possible to image the cortical blood flow at high resolution using laser speckle contrast imaging. And saline can recovery the skull to turbid instantaneously. Repeated experiments could be done in the next four weeks. It can be found that there are not any changes in cortical blood vessels or blood flow distribution. Therefore, it can be concluded that the skull optical clearing method enhances the contrast of both white-light and speckle images, which provides an innovative transparent cranial window for accessing cortical structural and functional information at high resolution with LSCI, and avoids the limitations of craniotomy-based cranial window with craniotomy. This study provides a switchable cranial window for imaging cortical blood flow.

8928-65, Session 13

Hemodynamic response studies using multimodal optical imaging technique in chronic epileptic mouse model

Hyuna Song, Areum Jo, Jeong-eun Sim, Sungkyunkwan Univ. (Korea, Republic of); Minah Suh, Sungkyunkwan Univ. (Korea, Republic of) and Samsung Advanced Institute for Health Science and Technology, Sungkyunkwan Univ. (Korea, Republic of); Beop-Min Kim, Sungkyunkwan Univ. (Korea, Republic of) and Korea Univ. (Korea, Republic of)

Spontaneous epileptiform discharges can be induced by intracortical injection of FeCl2 or FeCl3, which are known to cause chronic epileptogenesis in animals. In our previous study, we observed that total hemoglobin dynamics were altered in injection site of the brain which were monitored using two different optical imaging techniques including optical recording of intrinsic signal (ORIS) and near-infrared spectroscopy (NIRS).

In this study, hemodynamic signals in both superficial and deep region were recorded simultaneously using the same dual optical imaging methods. We also conducted electrophysiological recording of the local field potential (LFP) in order to classify seizure types into several groups based on the seizure onset and duration time.

Here, we analyzed the spike-related hemodynamic signals according to the epileptic events in each animal. Particularly, event-related NIRS and ORIS data were averaged respectively within each group and the grand averaged signal was synchronized with seizure onset time. We observed that hemodynamic response in ipsilateral hemisphere were significantly reduced and disrupted compared to contralateral hemisphere. Also, we performed causality analysis to investigate the direction and origin of hemodynamics in the disrupted brain. In addition, immunohistochemical data demonstrated that changes of GABAergic interneuron and astrocyte

in cortex can be correlated with our results. In summary, our multimodal optical imaging techniques can be utilized to investigate hemodynamic changes both in horizontal and vertical directions along with cortico-thalamic neural network in the whole brain.

8928-66, Session 14

Multiphoton 3D imaging and control of neurons *(Invited Paper)*

Darcy Peterka, Sean Quirin, Rafael Yuste, Columbia Univ. (United States)

We have recently coupled a volume projection imaging system to an LCOS-SLM based microscope to allow for three-dimensional modulation and capture of activity in neurons. The system relies on a simple, efficient phase-only wavefront coding element that allows for extended depth-of-field collection while maintaining the full numerical aperture of the microscope, and can be crafted to match the desired axial range required by the experiment. When combined with flexible three-dimensional deterministic multiphoton excitation afforded by our SLM microscope, we can modulate and image large numbers of neurons simultaneously in volumes with high temporal precision.

8928-67, Session 14

Astrocytic adaptation during cerebral angiogenesis follows the new vessel formation induced through chronic hypoxia in adult mouse cortex *(Invited Paper)*

Kazuto Masamoto, Iwao Kanno, National Institute of Radiological Sciences (Japan)

Neuro-glia-vascular unit (NGVU) plays a key role in maintaining functional integrity between central nervous system and cerebral microcirculation. Here, we examined the adaptive mechanisms of the NGVU during cerebral angiogenesis induced through chronic hypoxia. Using longitudinal two-photon microscopy and Tie2-GFP mice in which the vascular endothelial cells expressed green fluorescent proteins (GFP), the spatiotemporal evolution of the capillary sprouting and the neighborhood astrocyte remodeling labeled with sulforhodamine 101 were characterized over 3-week exposures to chronic hypoxia (8-9% O2). The functional integrity of the blood-brain barrier (BBB) and vascular responses to somatosensory whisker stimulation were also repeatedly examined. For early phases of the hypoxia adaptations (< 1 week), the soma and thin processes of the astrocytes remained unchanged, while the capillary sprouts were developed. In the later phase of the adaptations (> 2 weeks), capillary sprouts created new connections to the existing capillaries, while the neighborhood astrocytes extended their processes to the newly-formed capillaries. For an entire period of the hypoxia experiments, no leakage of the fluorescently labeled blood plasma was detected, indicating a preserved functional integrity of the BBB. In contrast, the arterial responses to whisker stimulation were gradually attenuated over the 3-week hypoxia exposures. These findings indicate that morphological adaptations of the astrocyte follow capillary development in the adult mouse cortex, while the functional integrity of the BBB is maintained by tight junctions of the endothelial cells. The attenuated vasodilatory responses of the arteries could represent a hypoxia-induced change of the neurovascular and/or gliovascular signaling.

8928-68, Session 14

Imaging neuronal activity using femtosecond laser pulses *(Invited Paper)*

Shaoqun Zeng, Huazhong Univ. of Science and Technology (China); Wei R. Chen, Univ. of Central Oklahoma (United States); Qingming Luo, Huazhong Univ. of Science and Technology (China)

Two-photon microscopy has grown up to be an important technique in biology research especially in the field of neuroscience for its high penetration depth and three-dimensional selectivity. However, the imaging rate of the conventional two-photon microscopy is limited by the mechanic scanning mechanism and cannot satisfy the requirement for imaging the encoding pattern of brunches of dendrite or cell populations in brain tissue. Laser scanning with two sequential orthogonally oriented (2D) acousto-optical deflectors (AODs) does not involve actual mechanical movement and thus provides a fast scanning rate, high precision, and stability. 2D AOD scanning also allows random access to each pixel in the field of view (FOV). Random scanning in regions of interest can devote dwell time to pixels of interest and increase the signal-to-noise ratio and the frame-capture rate, and would provide unique applications in neuroscience research. However this random scanning two-photon microscopy with femtosecond laser is frustrated by the temporal and spatial dispersion. With a special dispersion compensation scheme, we have constructed a random scanning two-photon microscope and are able to track the fast neuronal activity which is not available with other techniques. In this presentation, we will show 1) the evolution of the femtosecond laser pulse after passing the AOD scanner, 2) a technique to compensate the spatial and temporal dispersion simultaneously and facilitate two-dimensional random access two-photon microscopy, and 3) a special optimization algorithm which is particularly effective to reconstruct the firing pattern from calcium signal of low signal to noise ratio.

Conference 8928C: Optogenetics and Optical Control of Cells

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8928 Optical Techniques in Neurosurgery, Neurophotonics, and Optogenetics

8928-81, Session 15

The brain activity map: imaging the activity of entire neural circuits *(Invited Paper)*

Darcy Peterka, Rafael Yuste, Columbia Univ. (United States)

In physical systems built with many components, emergent properties, such as magnetism, are often generated from the interactions among these particles. These emergent properties are often invisible when observing individual particles, since they depend on large-scale interactions between them. Likewise, the function of the brain has been mostly studied by examining the responses of individual neuron, yet it is probably an emergent property that arises from the coordinated activity of large numbers of neurons in each of its neural circuits.

To capture this emergent level of brain function, we have launched a large-scale, international public project, the Brain Activity Map Project (or BRAIN Initiative), aimed at developing new methods to measure and control neural activity across complete neural circuits in experimental animals and human patients. This technological effort will be an interdisciplinary project, incorporating into neuroscience many methods and approaches from the physical sciences and nanotechnologies. The data obtained with these new methods could prove to be an invaluable step towards understanding fundamental and pathological brain processes. Finally, the novel technologies developed by this project, like it happened with the Human Genome Project, could give rise to new areas of economic and industrial development.

8928-82, Session 15

3D optrode array neural interface for comprehensive and/or selective optogenetic light delivery

Tanya Vanessa F. Abaya, Mohit Diwekar, Steve Blair, Loren Rieth, Prashant Tathireddy, Florian Solzbacher, Univ of Utah (United States)

We present transmission and illumination characteristics of needle-type waveguides in neural arrays for optogenetic light delivery in deep tissue. The waveguide arrays were micro-machined from fused silica wafers. Each waveguide, called an optrode, can transmit light with >90% efficiency into >1 mm deep below tissue surface; light attenuation is tissue is mitigated by 3 orders of magnitude. Emitted profiles in tissue have divergence values in the range of 13 – 40° for tip internal angles of 30 – 45° with respect to the light propagation direction. Initial output beam sizes closely follow the optrode width, which is 70 – 150 ?m in this case. The beam characteristics may satisfy a wide range of applications for targeted optogenetic illumination.

8928-83, Session 15

Integrated neural probe for localized light delivery and electrical recording

Wei-Chuan Shih, Mufaddal M. Gheewala, Univ. of Houston (United States); Gopathy Purushothaman, Vanderbilt Univ. (United States); John A. Dani, Univ. of Pennsylvania (United States); John C. Wolfe, Univ. of Houston (United States)

Optogenetics enables molecular specific stimulation of neuronal action potential firing by activating channelrhodopsin 2 (ChR2). Coupled with microelectrode spike recording, a “feed back” control system has been envisioned. However, separate optical delivery and electrical recording probes are typically employed in existing demonstrations. Other devices based on integrated waveguide technology suffer from low light delivery efficiency due to coupling and propagation loss.

Recently, we have proposed the integration of microelectrodes and wirings directly on a thin optical fiber of diameter ~60 ?m. This approach drastically departs from building integrated waveguides and electrode arrays on planar substrates, or bundling optical fiber with metal microwires. Since high-quality optical fibers are readily available, robust and high-efficiency light delivery is expected. However, to pattern microelectrodes and wirings on a curved substrate is challenging for conventional lithography technology. We have developed a microfabrication suite for depositing and patterning microstructures based on vacuum thin film processes and ion/atom beam lithography. We have fabricated integrated neural probes with one optical delivery port and 4 surrounding microelectrodes, similar to the configuration of a twisted-wire tetrode. We will show preliminary characterization of light delivery efficiency and impedance measurements. We will also show stimulation and recording results from in vivo experiments.

8928-84, Session 16

Development of new optogenetic tools: red light activatable channelrhodopsin and inhibitor of synaptic release *(Invited Paper)*

John Y. Lin, Per Magne Knutsen, Arnaud Muller, Sharon B. Sann, Keming Zhou, Sayyed Nabavi, Christophe Proulx, David Kleinfeld, Roberto Malinow, Univ. of California, San Diego (United States); Yishi Jin, Roger Y. Tsien, Univ. of California, San Diego (United States) and HHMI (United States)

In the current study, we described the development and utilization of two novel optogenetic tools: a Red-activatable Channelrhodopsin (ReaChR), that is optimally excited with orange to red light, and an optogenetic inhibitor of presynaptic vesicular release.

By combing chimeragenesis and point mutation, we were able to generate a new variant of channelrhodopsin that responds robustly to red-orange and red light (590 - 635 nm). When expressed in vivo in layer Vb of vibrissae motor cortex and facial nucleus in the brainstem, we were able to evoke whisker movement non-instrusive through intact skin, bone and brain tissue with externally placed LED.

Separately, we have used chromophore assisted light inactivation (CALI) to inhibit synaptic release by fusing a genetically-encoded singlet oxygen generator, miniSOG, to proteins involved in synaptic vesicular release. Upon light illumination, we were able to efficiently inhibit synaptic release in cell culture and organotypic slices. When expressed in vivo in C. elegans and rat, we were able to inhibit movements in the worms and modify behavior in the rat. We named this technique Inhibition of Synapses with CALI (InSynC).

We believe both of these tools have potentials to be utilized in various neurobiological application and further expands the optogenetic tools available for researchers.

8928-85, Session 16

Non-invasive activation of optogenetic actuators

Elisabeth Birkner, Duke University (United States); Ken Berglund, Duke Univ. (United States); Marguerita E. Klein, Duke University (United States); George J. Augustine, Lee Kong Chian School of Medicine, Nanyang Technological University (Singapore); Ute Hochgeschwender, Duke Univ. (United States)

The manipulation of genetically targeted neurons with light (optogenetics) continues to provide unprecedented avenues into studying the function of the mammalian brain. However, potential translation into the clinical arena faces a number of significant hurdles, foremost among them the need for insertion of optical fibers into the brain to deliver light to opsins expressed on neuronal membranes. In order to overcome these hardware-related problems, we are proposing an alternative strategy for delivering light to opsins which does not involve fiber implants. Rather, the light is produced by a protein, a luciferase, which oxidizes intravenously applied substrate, thereby emitting bioluminescence. In proof-of-principle studies employing a fusion protein of a light-generating luciferase to a light-sensing opsin (luminopsin) we showed that Gaussia luciferase-emitted light is indeed able to activate channelrhodopsins, allowing modulation of neuronal activity when expressed in cultured neurons. We since have generated a variety of fusion constructs with different combinations of luciferases and opsins. These include pairing Gaussia luciferase variants emitting higher luminescence with channelrhodopsins with higher light sensitivity, as well as with proton pumps (ArchT, Mac). Using a mutant version of Gaussia luciferase which emits higher levels of bioluminescence in fusions with channelrhodopsins and proton pumps resulted in action potential firing and silencing, respectively, in primary neurons in vitro. Thus, luminopsins allow both excitation and inhibition of neurons through non-invasively activated bioluminescence. Further developments of such technology based on combining optogenetics with bioluminescence, i.e. combining light-sensing molecules with biologically produced light through luciferases, might bring optogenetics closer to clinical applications.

8928-86, Session 16

Optogenetically controlled neural stem cell differentiation

Young-tae Kim, Shahina Ahmed, Samik Bhattarai, Kamal Dhakal, Bryan Black, Samarendra K. Mohanty, The Univ. of Texas at Arlington (United States)

Spatial control of neural stem cells (NSCs) differentiation into neurons, astrocytes and oligodendrocytes in defined time-points has potential for treatment of neuro-degenerative diseases such as Alzheimer's, Parkinson's and Multiple sclerosis. Significant difficulties exist in confining application of chemical cues to specific cells and regions in a timely-controlled manner. Optogenetic stimulation has been shown to cause membrane depolarization, which plays an important role in embryonic stem cell differentiation (ESCs). Here, we report use of microbial opsins introduced into NSCs, followed by optogenetic excitation for noninvasive modulation of NSC differentiation. Mouse NSCs were transfected with opsin-marker fluorescent protein using viral vectors. Excitation of opsin-expressing NSCs with light of varying dose and frequency resulted in modulation of their electrical activity, measured by multi-electrode array (MEA). Differentiation of these excited-NSCs was assessed by expression of mature neuronal proteins and morphology. Optimal parameters of optogenetic stimulation for effective differentiation were determined. Potential uses of this technology for noninvasive optical control of NSC differentiation in in-vitro as well as in-vivo environments will be presented.

8928-87, Session 16

A versatile and low cost stimulation source for optogenetics experiments

Frederic Pain, Farnoosh Farmani, Univ. Paris Sud (France) and CNRS (France); Maud Marty, Univ. Paris Sud (France); Eric Marty, PHYMEP (France); Serge Luquet, Univ. Paris 7-Denis Diderot (France); Claire Martin, Univ. Paris Sud (France)

Optogenetics experiments require pulsed light sources, with a spectrum matching with the Channel-Rhodopsin-2 absorption spectrum. Approaches using filtered arc lamp, laser diodes or high power light emitting diode have been successfully implemented, some being commercialized. Here we propose an alternative versatile and low cost system based on a pigtailed high power led associated to an Arduino microcontroller board used as a pulse generator. Available power per mm square was measured using a power meter and the actual light pulse characteristics were assessed using a photomultiplier. Several high flux and high power leds were tested. About 5mW/mm? of light power at 465nm was obtained at the tip of a 100µm diameter multimode glass fiber. Full control of the pulse train characteristics (frequency, duty cycle, duration) is available either via software or via analogue controls. The light stimulation pattern can be highly customized, with repeating patterns or single stimulation triggered by external events, i.e. a 5V TTL signal. Light intensity can also be controlled in order to vary the amount of cells being excited. Monte Carlo simulations of light transport were carried out in 3D in order to investigate this effect.

8928-101, Session 16

Transplantation of retinal progenitor cells for retinal repair *(Invited Paper)*

Henry J. Klassen M.D., Univ. of California, Irvine (United States)

Transplantation of retinal progenitor cells (RPCs) to the degenerating retina has revealed the therapeutic potential of this approach in the setting of currently untreatable blinding diseases. The mechanism of action can involve integration into the host retina and differentiation into retinal cell types such as photoreceptors or, alternatively, efficacy can result from trophic-mediated neuroprotection of host photoreceptors. At this time, much has been done to distinguish the RPC as a significant therapeutic candidate, including GMP production of human RPCs. There is much to recommend this approach, including simplicity, safety, and particularly the potential for cell-mediated efficacy in currently untreatable blinding diseases.

8928-88, Session 17

Optical control of cells: past, present, and future *(Invited Paper)*

Michael W. Berns, Univ. of California, Irvine (United States) and Univ. of California, San Diego (United States)

The first use of photons to alter and manipulate live cells occurred in 1912 when the Russian Sergej Tschachotin created a unique optical microscope that could focus 280 nm UV light to a near-diffraction spot and alter parts of invertebrate cells and embryos. That basic technology is still used by biologists today, but the introduction of the 694.3 nm ruby laser microbeam by Marcel Bessis and his colleagues in Paris in 1962 opened the door for far more precise spatial and temporal control of cell and subcellular optical manipulation. The first application to genetic manipulation (before the term "optogenetics" was coined) was published in 1969 by Donald Rounds and I using the 532 and 488 nm pulsed argon ion laser. Over the past forty years the pulsed 532 nm second harmonic Nd:Yag (ns and ps) and more recently the femtosecond

NIR Titanium sapphire lasers have been used by many labs to alter DNA in chromosomes, study the DNA repair process, alter gene expression using single and multiphoton absorption, and as stated in the description of this conference on "optogenetics," combined genetic and optical methods has allowed controlled (stimulation or silencing) of electrically-activatable, genetically-targeted cells with high temporal precision. It is clear that optical manipulation of single or groups of cells in vitro and/or in vivo will be an important research area for years to come, and should result in significant clinical outcomes.

8928-89, Session 17

Force, flow and heat: optical control of axonal guidance *(Invited Paper)*

Samarendra K. Mohanty, The Univ. of Texas at Arlington (United States)

The human brain, the most mysterious phenomenon in the universe, is a highly complex system containing nearly hundred billions of neurons and trillions of connections. Neural signaling, transmitted and integrated through these complex connections, is responsible for thousands of specialized functions that control every aspect of our lives. The establishment of functional connections via axonal pathfinding is dynamic in nature and continues from neurogenesis until adulthood. While advancement has been made in understanding axonal guidance by different chemical cues, there has been a lack of understanding if and how axons respond to physical cues. We have discovered how different physical cues such as force, flow, and heat etc can be used to guide axons with varying efficacy. These cues can be very effectively generated by photonic means in a spatio-temporally localized and controlled manner. This talk is focused to describe our integrated approach towards understanding of neural response to light-generated physical cues. Use of these optical guidance technologies to unravel unique properties of axons will be presented.

8928-90, Session 18

Photochemically controlled pathway for cellular migration

Young-tae Kim, Samik Bhattarai, Shahina Ahmed, Bryan Black, Samarendra K. Mohanty, The Univ. of Texas at Arlington (United States)

Controlling migration of cells and cellular processes is vital for successful tissue repair after injury. Use of chemical cues such as growth factors for cellular guidance is limited by lack of high spatial and temporal control, especially in in-vivo environments. Further, role of stiffness of microenvironment on cellular migration is not fully understood. We hypothesized that physical cue such as gradient in stiffness of microenvironment can modulate polarization of cells for guided migration. Here, we report creation of stiffness variation in microenvironment of cells using optical control. This involved use of photo-initiator and focused ultrafast near-infrared laser microbeam. By use of the two-photon process, high spatial and temporal control in forming pathway of varying stiffness could be achieved in Hyaluronic acid hydrogels containing different % of photo-initiators. Measurement of stiffness of the light-transformed microenvironment and interaction of various types of cells with the light-created pathways will be presented.

8928-91, Session 18

Spatial light modulation and imaging system for investigation of neurometabolic and neurovascular coupling

Ryan Baumgartner, Univ. of Wisconsin-Milwaukee (United States); Thomas J. Richner, Sarah Brodnick, Justin Williams, Kevin W. Eliceiri, Univ. of Wisconsin-Madison (United States); Ramin Pashaie, Univ. of Wisconsin-Milwaukee (United States)

We developed a spatial light modulator (SLM) and imaging system for in vivo optogenetic investigation of neurometabolic and neurovascular coupling. We report optogenetic modulation of neural potentials, metabolic signals, and blood vessel diameters. Our approach utilizes a digital micromirror device (DMD) microprojector to pattern light onto the cortex of a transgenic mouse whose pyramidal neurons are activated when exposed to blue light. To gain optical access to the cortex, a small area of the cranium is replaced with a coverslip, creating a window into the brain. We also incorporated epi-fluorescence optics and CCD camera into the optical platform. Fluorescence imaging time-series of cerebral blood vessels before and after applying brief photostimulus patterns with the SLM revealed optogenetically induced vasodilation of the branches of the middle cerebral artery (MCA) of up to a 30% increase in diameter, depending on stimulation area. Veins in the same region showed less response as expected. This result provides direct evidence of an optogenetically mediated hemodynamic response and is consistent with previously reported optogenetic modulation of the fMRI BOLD signal. To investigate neurometabolic coupling, we imaged nicotinamide adenine dinucleotide (NADH), an endogenous fluorescent enzyme involved in the Krebs cycle. NADH fluorescence intensity decreased transiently following optogenetic stimulation, indicating increased oxidation. Having investigated hemodynamics and metabolism, we next verified spatial neural activation by recording cortical potentials. We microfabricated electrocorticography electrode arrays on a transparent polymer and implanted them under the cranial window so that we could record neural potentials while still having optical access for the microprojector.

8928-92, Session 18

Fiber optic fluorescence microscopy for functional brain imaging in awake mobile mice

Jaepyeong Cha, Martin Paukert, Dwight E. Bergles, Jin U. Kang, Johns Hopkins Univ. (United States)

Understanding the causal relationships between cellular activity in the brain and animal behavior and is a central goal in neuroscience. Electrophysiological approaches have been used to study the firing patterns of neurons in freely moving animals, but little is known about the activity of electrically silent cells such as astrocytes. However, in concert with recently developed fluorescent markers, optical methods now permit minimally invasive and comprehensive sampling from both neuronal and non-neuronal cells in awake animals. In addition, fiber-optic technology has expanded the imaging capability into freely behaving animals. These advantages mainly stem from optical fiber's compactness, accessibility and flexibility.

In this work, we present a novel approach to detect functional brain activity in live mice with minimal invasiveness using fluorescence fiber-optic microendoscopy. The system uses a flexible endoscopic probe composed of a 30,000 core, 650-micron-diameter coherent fiber-bundle and an approximately 1500-micron working distance miniature objective. The fiber-optic neural interface at the distal end of the probe can be mounted to a 4-mm2 cranial window without touching brain surface, allowing imaging of locomotion-induced calcium transients in Bergmann glial cells in mice that express the genetically encoded calcium indicator GCaMP3. To the best of our knowledge, this is the first demonstration of

fiber-optic glial calcium imaging in the cerebellum of awake, mobile mice. We evaluated the performance of this system through in vivo studies by imaging Bergmann glia in head-fixed mice walking on a treadmill. Our configuration is not limited to head-restrained mice, but is also well suited for freely behaving animal models.

8928-93, Session 18

Impact of near-infrared laser irradiation on neuronal growth

Amit Kumar, The Univ. of Texas at Arlington (United States) and Indian Institute of Science Education and Research Kolkata (India); Raghave Upadhyaya, Kamal Dhakal, Samarendra K. Mohanty, The Univ. of Texas at Arlington (United States)

Near-infrared (NIR) pulsed laser beam has been used in the past for multi-photon imaging, stimulation, optoporation as well as axotomy of neuronal processes based on the pulse energy. Further, NIR cw laser beam is finding use for control and guide axonal growth and for phase sensitive measurements of neuronal activity. More interestingly, at low dose, it has been shown that low level NIR irradiation of neurons leads to increased cortical ATP, reduced oxidative stress and improved neurological recovery. Here, we report study of neuronal growth as a function of different NIR laser irradiation parameters. Time-lapse imaging of neurons was performed in an environmentally-controlled environment on an inverted microscope platform. In order to quantify growth and changes in small time scales, several image processing steps were carried out. This includes contrast enhancement, image registration, image stabilization, defining ROI, area detection, edge detection, thresholding, dilation, erosion and differential imaging. While at higher cw NIR intensities, retraction of growth cone was observed, at low intensities enhancement of growth rate was achieved. Irradiation of distal end (growth cone) of axon by ultrafast scanning NIR laser beam at low intensities also led to enhancement of the growth rate. Optimal laser irradiation parameters (e.g. power) for enhancing growth rate and plausible mechanisms will be presented.

8928-102, Session 18

Potential applications of optogenetics for aging and Alzheimer's research

Wen G. Chen, National Institutes of Health (United States)

No Abstract Available

8928-94, Session 19

Molecular reaction mechanism of channelrhodopsin *(Invited Paper)*

Klaus B. Gerwert, Ruhr-Univ. Bochum (Germany)

Channelrhodopsin is the crucial tool in optogenetics. In order to elucidate the molecular reaction mechanism time-resolved FTIR spectroscopy in combination with biomolecular simulations (MD and QM/MM) is applied as before to bacteriorhodopsin. (1). Protein bound water molecules conduct the proton with in Bacteriorhodopsin (2,3). This approach is recently extended to channelrhodopsin (4). Deprotonation of E-90 is identified as crucial gate for water invasion and channel formation, and proposed to be the key residue in the channel selectivity mechanism. Using ns-Step scan FTIR in combination with biomolecular simulations we elucidate the molecular reaction mechanism of channelrhodopsin. Reorientation of protein bound water molecules is the key. This insight will allow us to taylor the next generation channelrhodopsins for optogenetic applications.

8928-95, Session 19

Regulating cofilin spatiotemporal dynamics and transport using optogenetic modulation

Atena Zahedi, Univ. of California, Riverside (United States); Seyyed Farhad Razavi, Consultant (United States); Vincent On, Iryna Ethell, Univ. of California, Riverside (United States)

Neurodegenerative disorder Alzheimer's disease (AD) is characterized by the abnormal accumulation of pathogenic ?-amyloid (A?)1-42 peptides and formation of neuritic plaques in the brain, which lead to progressive cognitive decline. Early stages of learning and memory decline in AD patients are associated with a pronounced loss of synapses, but mechanisms underlying synaptic loss prior to the formation of plaques are yet unclear. In particular, excessive activation of actin-severing protein cofilin has been implicated in A?-mediated loss of dendritic spines and synapses. However, little is known about the spatiotemporal dynamics of cofilin activation and its transport in synapses. Here, we describe a novel approach that combines optogenetics and multi-channel live imaging system to track cofilin, regulate its activity using photoactivatable probes, and modulate synaptic connectivity in cultured hippocampal neurons. We performed simultaneous imaging and activation of a photoactivatable Rac1 (PA-Rac) probe in cultured hippocampal neurons, which suppressed cofilin activity through a Pak1-LIMK1-mediated pathway. In a series of live 2-photon imaging experiments, we showed that phosphorylation/ inactivation of cofilin with PA-Rac triggers its export from dendritic spines. The spatiotemporal dynamics were analyzed using several live video segmentation program designed to monitor the changes in cofilin localization, actin remodeling response, and the morphology of dendritic spines. a Comsol model was developed to simulate synaptic changes in the video data and compare against experimental data. By modulating the dynamics of cofilin, we hope to shed light on potential therapies that aim to restore loss of synaptic connections and functions in AD.

8928-96, Session 19

Recombinant Adeno-associated virus (rAAV)-mediated transduction and optogenetic manipulation of cortical neurons in vitro

Wienke Lange, Lei Jin, Forschungszentrum Jülich GmbH (Germany); Vanessa Maybeck, FZ Jülich, Institute of Complex Systems, Bioelectronics (ICS-8) (Germany); Annika Meisenberg, Forschungszentrum Jülich GmbH (Germany); Arnd Baumann, FZ Jülich, Institute of Complex Systems, Cellular Biophysics (ICS-4) (Germany); Andreas Offenhäusser, Forschungszentrum Jülich GmbH (Germany)

Optogenetic or genetically encoded light-sensitive proteins can be used to manipulate and observe cellular functions. According to these different modes of action they are divided into actuators like the blue-light gated cation channel Channelrhodopsin-2 (ChR2) and detectors like the Calcium sensor GCaMP3.

In this project, we want to optogenetically control and study the neuronal activity of rat primary cortical neurons in vitro. Therefore, the light-stimulation of ChR2-expressing neurons will be combined with electrophysiological methods like patch clamp and multielectrode arrays as well as with optical read-out systems. To specificially express the optogenetic proteins in different neuronal cell types, we use recombinant Adeno-associated viruses (rAAVs) as gene-ferries together with cell-specific promoters.

Several rAAV serotypes were tested concerning the efficiency and neuronal specificity of the transduction of cortical neurons in neuronal-glial mixed cultures. We used the expression of GFP under the control of a CMV promoter as a first read-out system. Our results showed the

best efficiency for the transduction with serotype 6. With our established rAAV transduction procedure we expressed different transgenes with high expression rates in neurons as well as in glial cells. Using the human synapsin I promoter, we were able to limit the expression to neuronal cells.

The genetically encoded Calcium indicator GCaMP3 was used to measure the neuronal activity through Calcium imaging of cortical neuronal cultures. Action potentials of ChR2-expressing neurons could be repeatedly and precisely triggered with blue light laser pulses and be measured through patch clamp experiments.

8928-97, Session 19

Four-state photocycle modelling of two-photon optogenetic activation

Amit Kumar, The Univ. of Texas at Arlington (United States) and Indian Institute of Science Education and Research Kolkata (India); Ling Gu, Samarendra K. Mohanty, The Univ. of Texas at Arlington (United States)

Optical stimulation of genetically-targeted cells has evolved as an effective tool for the study of cellular systems, both in-vitro and in-vivo. In contrast to conventional single-photon optogenetic methods, we introduced two-photon optogenetic stimulation (TPOS) using near-infrared (NIR) laser beam to enable higher spatial precision by virtue of the nonlinear nature of ultrafast light interaction with the opsins. In addition, TPOS allows deeper penetration due to low scattering and absorption coefficients of tissue in the NIR spectral region. Recently, we reported development and implementation of non-scanning fiber-optic TPOS (FO-TPOS) of channelrhodopsin-2 (ChR2)-transfected excitable cells replacing the original use of microscope objective and scanning two-photon laser beam. It may be noted that until now the two-photon cross section of ChR2 has been estimated based on a two-state model. Here, we report development of a four-state model for TPOS of ChR2 and present detailed study of the photocurrent arising from the FO-TPOS. This includes estimation of the ChR2-activation cross-section at various intensities and wavelengths. We will also outline the contributions to the net photocurrent by different potential phenomena that may arise during NIR stimulation.

8928-98, Session 20

Effect of light stimulation on interictal spikes from ChR2 expressed CA1 pyramidal cells

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Interictal spikes (IISs) result from abnormal synchronization of neuronal discharges. The phenomenon of IISs has been of keen interest to researchers in the recent years because of their debatable role in the onset of epilepsy. Furthermore, there has been some evidence suggesting that IISs affects long-term cognitive performance. In this context, having the capability of controlling IISs in an epileptic brain, may facilitate a better understanding of IISs and their role in subsequent ictal activity. However controlling IISs would require the control of synchronization of paroxysmal depolarization shifts (PDS), the cellular correlates of IIS, consisting of pathological bursts from excitatory hippocampal pyramidal neurons. This may require exhaustive trial-and-error experiments in an in vivo setting. We simplify the development of IIS control using computational models of optogenetically activated/suppressed neurons in a bio-physically detailed CA1 network model. First we model channelrhodopsin (ChR2) and halorhodopsin (NpHR) opsins within our CA1 pyramidal neuron models. Next, we use a CA1 network model that produces IISs in the absence of light. The CA1 network exhibits IISs in a variety of morphological conditions such as increased recurrence between pyramidal cells, and variability in input from the Schaffer collaterals. We investigate various light stimulation protocols that disrupt synchronization of PDSs. Finally, we study the relationship between the morphological changes in CA1 and its sensitivity to light input parameters for the disruption of IIS. Our findings shed some light on the design of effective optogenetic stimulation protocols to effectively control IIS in vivo.

8928-99, Session 20

A system for combined in vivo cellular resolution optogenetic stimulation and imaging for vision research *(Invited Paper)*

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Optical retinal prostheses for patients with outer-retinal degenerative diseases could interface directly with surviving retinal neurons in order to emulate a meaningful percept in the brain. Recently, we introduced an artificial photo-stimulation technique based on the projection of holographic patterns with high spatiotemporal resolution onto optogenetic probes, for selectively controlling large retinal neuronal populations in isolated mouse retinas.

Here, we present a system for targeting multiple optogenetic-expressing rodent retinal ganglion cells (RGCs) in-vivo with holographic patterns at a cellular resolution, while imaging the response in downstream circuits using calcium probes expressed in the visual cortex. The system combines precise funduscope-guided spatiotemporal holographic photo-stimulation with a multiphoton microscope that enables cortical functional imaging of the responses to artificial stimulation. The funduscope brightfield and fluorescence images in mice and rats enable the identification of single fluorescent RGCs for stimulation using holographic patterns with spot diameters sufficient for cellular targeting.

The new integrated system combines high spatial and temporal resolution artificial visual stimulation together with multiphoton functional calcium imaging of neuronal populations at a single-cell resolution (using indicators like GCaMP6), allowing the monitoring of responses to artificial stimulation in blind optogenetic animals, and the investigation of network dynamics in response to highly controlled visual stimuli in the healthy visual system.

8928-100, Session 20

Mueller matrix polarimetric imaging of neurons

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Optical techniques have not only enabled structural imaging of fluorescently-labelled neurons, but also allowing functional imaging by use of biochemical probes such as calcium and voltage-sensitive dyes. However, there is a growing demand for label-free quantitative optical imaging of neurons. While quantitative phase microscopy has been used for imaging neuronal processes, phase-change introduced by the sample may not be ideal for extracting important information about the neurons

and their activity. In order to extract other important optical properties of neurons such as diattenuation and birefringence, we attempted Mueller matrix (MM) based polarimetric imaging of neurons. The polarimetric microscopic imaging setup was built on an inverted microscope platform and integrated with a CCD camera for mapping the MM elements in neurons. The setup utilized liquid crystal variable retarders with polarizers and quarter-waveplates to generate and analyze the polarization states of the light incident/transmitted through the neurons. Time-lapse MM-based polarimetric imaging of neurons (PC12) was performed in an environmentally-controlled environment. Measurement of MM-maps provided diattenuation, birefringence and depolarization maps of the growing neurons. We believe that some of these constituent polarization properties, if properly extracted and quantified, can potentially serve as useful biophysical and physiological metric for detecting neuronal activities.

8929-1, Session 1

Characterization, diagnosis and ablation of human teeth using blue laser at 457 nm.

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Absorption, scattering and fluorescence together determine the color of human teeth. These properties can probably be used as the basis of quantitative diagnostic methods for caries. It is useful therefore to quantitate these properties when possible. The absorption and scattering coefficient of human teeth were determined from detached wet teeth, Human incisors and human premolars were used. Absorption, transmission and reflection of these teeth were measured using compact blue laser source at wavelength of 457 nm and a high resolution spectrometer equipped with an integrating sphere. The average absorption coefficient of abnormal tissue of human teeth is higher than the normal ones. Detection and diagnosis of caries tissues with high accuracies were monitored by high resolution translational scanning of human teeth. We have a powerful tool to diagnosis a caries region of human teeth using blue laser at 457 nm. Ablations of caries region are investigated using higher power blue laser at 457 nm. The experiments confirmed that this module provides efficient tissue incision performance and faster healing.

8929-2, Session 1

Does ozone enhance the remineralizing potential of nanohydroxyapatite on artificially demineralized enamel? A laser induced fluorescence study

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The present era of minimal invasive dentistry emphasizes the early detection and remineralization of initial enamel caries. Ozone has been shown to reverse the initial demineralization before the integrity of the enamel surface is lost. Nanohydroxyapatite is a proven remineralizing agent for early enamel caries. In the present study, the effect of ozone in enhancing the remineralizing potential of nanohydroxyapatite on artificially demineralized enamel was investigated using laser induced fluorescence. Thirty five sound human premolars were collected from healthy subjects undergoing orthodontic treatment. Fluorescence was recorded by exciting the mesial surfaces using 325 nm He-Cd laser with 2 mW power. Tooth specimens were subjected to demineralization to create initial enamel caries. Following which the specimens were divided into three groups, i.e ozone (ozonated water for 2 min), without ozone and artificial saliva. Remineralization regimen was followed for 3 weeks. The fluorescence spectra of the specimens were recorded from all the three experimental groups at baseline, after demineralization and remineralization. The average spectrum for each experimental group was used for statistical analysis. Fluorescence intensities of Ozone treated specimens following remineralization were higher than that of artificial saliva, and this difference was found to be statistically significant ($P<0.0001$). In a nutshell, ozone enhanced the remineralizing potential of nanohydroxyapatite, and laser induced fluorescence was found to be effective in assessing the surface mineral changes in enamel. Ozone can be considered an effective agent in reversing the initial enamel caries there by preventing the tooth from entering into the repetitive restorative cycle.

8929-3, Session 1

Guided fluorescence diagnosis of childhood caries: preliminary measures correlate with depth of carious decay

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The current rise in childhood caries worldwide has increased the demand for technologies that can quickly and accurately detect early stage carious lesions. These lesions, if detected at an early stage, can be reversed with remineralization treatments and improvements in home care. A multi-modal optical prototype for diagnosing occlusal caries demineralization in vivo has been developed and pilot tested. The device uses a 405-nm laser as a scanned illumination source to obtain high resolution and high surface contrast reflectance images, which allows the user to quickly image and screen for any signs of demineralized enamel. When a suspicious region is located, the device can be switched to perform dual laser fluorescence spectroscopy using 405-nm and 532-nm laser excitations. These spectra are used to compute an auto-fluorescence (AF) ratio of the suspicious region and the percent difference of AF ratios from a healthy region of the same tooth. The device was tested on 6 children's teeth in vivo with clinically diagnosed carious lesions. Lesion depth was then visually estimated from the video image using the 405-nm scanned illumination source, and within a month later the maximum drill depth was assessed by a clinician. The researcher and clinicians were masked from previous measurements in a blinded study protocol. Preliminary results show that the ratiometric percent difference measurement of the AF spectrum of the tooth correlates with the severity of the demineralization as assessed by the clinician after drilling.

8929-4, Session 1

Viability of imaging structures inside human dentin using dental transillumination

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Dental Transillumination (DT) is a technique for imaging internal structures of teeth by detecting infrared radiation transmitted throughout the specimens. It was successfully used to detect caries even considering the strong scattering of dental enamel and dentin on infrared radiation region. Literature reports that enamel's scattering coefficient is 10 to 30x lower than dentin; this explains why DT is useful for imaging pathologies in dental enamel, but does not disable its using for imaging dental structures or pathologies inside the dentin. Until now, there was no conclusive data in the literature about the limitations of using DT to access dentin information.

Here is presented a DT application to imaging internal structures of dentin. Tooth slices were confectioned varying their thickness in different groups. For imaging a FPA InGaAs camera model Xeva 1.7-320 (900nm-1700nm; Xenics, Inc., Belgium) and a 3W lamp-based broadband light source (Ocean Optics, Inc., USA) were used; bandpass optical filters at 1000nm±10nm, 1100nm±10nm, 1200nm±10nm and 1300nm±50nm spectral region were also used to spectral selection of imaging. Spectral images were captured using different sensor exposures (camera calibration) with and without the filters. A computational processing

was applied to spectral images obtained. The best results revealed the viability to imaging dentin tissue with thickness of up 1mm at 1300nm±50nm and up 3.0mm without a filter (900-1700nm: spectral range). Once with this results a pilot experiment was made using DT to detect the pulp chamber of an incisive human tooth and its viability was proved to imaging the specimen pulp chamber.

8929-5, Session 1

SOPROCARE - 450 nm wavelength detection tool for microbial plaque and gingivitis: a clinical study

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Gingivitis due to microbial plaque and calculus can lead over time if left untreated to advanced periodontal disease with non-physiological pocket formation. Removal of microbial plaque in the gingivitis stage typically achieves gingival health.

The SOPROCARE camera system emits blue light at 450 nm wavelength using three blue diodes. The 450 nm wavelength is located in the non-ionizing, visible spectral wavelength region and thus is not dangerous. It is assumed that using the SOPROCARE camera in perio-mode inflamed gingiva can easily be observed and inflammation can be scored due to fluorescence from porphyrins in blood. The assumption is also that illumination of microbial plaque with blue light induces fluorescence due to the bacteria and porphyrin content of the plaque and thus can help to make microbial plaque and calculus visible.

Aim of the study with 55 subjects was to evaluate the ability of the SOPROCARE fluorescence camera system to detect, visualize and allow scoring of microbial plaque in comparison to the Turesky modification of the Quigley and Hein plaque index. A second goal was to detect and score gingival inflammation and correlated the findings to the Silness & Löe Gingivitis Index.

The study showed that scoring of microbial plaque as well as gingival inflammation levels similar to the established Turesky modified Quigley Hein index and the Silness & Löe Gingival Inflammation index can easily be done using the SOPROCARE system in perio-mode.

Linear regression fits between the different clinical indices and SOPROCARE scores in daylight and fluorescence mode as well as comparisons to digital photography revealed the system's capacity for effective discrimination between scores.

8929-6, Session 2

Structure and chemical composition of the dentin-enamel junction analyzed by Confocal Raman Microscopy

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The structure and chemical composition of the human dentine-enamel junction (DEJ) was studied using confocal Raman microscopy - a chemical imaging technique. Slices of non-fixed, sound teeth were prepared with an Isomet diamond saw and scanned with Witec Alpha300R system.

The combination of different characteristics peaks of phosphate, carbonate and organic matrix (respectively 960, 1072 and 1545 cm-1), generates images representing the chemical composition of the DEJ area. Images are also calculated using peak ratios enabling precise determination of the chemical composition across the DEJ. Then, with two characterized peaks, different pictures are calculated to show the ratio of two components. The images of the spatial distribution of mineral phosphate (960cm-1) to organic matrix (1545 cm-1) ratios, mineral carbonates (1072cm-1) to mineral phosphate ratios; and mineral carbonates to organic matrix ratios were reconstructed. Cross sectional and calculated graphic profile show the variations of the different chemical component ratios through the enamel and the dentine.

Phosphate to organic ratio shows an accumulation of organic material under the enamel surface. The cross sectional profile of these pictures shows a high phosphate content compared to enamel in the vicinity of the DEJ.

The Confocal Raman imaging technique can be used to further provide full chemical imaging of tooth, particularly of the whole DEJ and to study enamel and dentine decay.

8929-7, Session 2

Ablation of human carious dentin with a nanosecond pulsed laser at a wavelength of 5.85 µm: relationship between hardness and ablation depth

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Less-invasive treatment and preservation of teeth, referred to as minimal intervention, is a strong requirement in dentistry. In this regard, the use of Er:YAG laser and Er,Cr:YSGG laser has already been used because they realize comfort treatments due to the lack of noise or vibration. However, it has been reported that these lasers sometimes exhibit poor selectivity for caries removal. Therefore, there is a need to develop new laser techniques for the selective ablation of caries in minimal intervention. In our previous studies, the fundamental ablation properties of dentin at wavelengths of 5.6–6.6 µm were investigated. The selective ablation of demineralized dentin with minimal damage to sound dentin at wavelengths around 5.8 ?m and the easy control were shown. Also, the wavelength of 5.85 ?m has been a candidate for the selective ablation of human carious dentin. However, the mechanism of the ablation selectivity is unclear. The purpose of this study was to investigate the ablation property and the relationship between hardness of human carious dentin and the ablation depth by a nanosecond pulsed laser irradiation. As a result, it was indicated that the ablation depth for demineralized dentin was greater than that for sound dentin because the large ablation was observed in low Ca content. Evaluating hardness before irradiation is necessary to control the selective removal of carious dentin according to the stage of caries progression in minimal intervention dentistry.

8929-8, Session 2

Er:YAG laser delivery sytems and sonic-activated bulk composite restoration: sculpturing and microleakage evaluation

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Dental enamel and dentin contain sufficient water volume, and thus, radiation generated by mid-IR Er-based laser system can achieve effective ablation. The aim of this study was to compare the tissue quality and its restoration after contact, non-contact, and non-contact scanned Er:YAG laser radiation ablation. Laser setting for contact ablation was 250 mJ/pulse, pulse repetition rate 15 Hz, average power 3.75 W. For

non-contact ablation these values were 600 mJ/pulse, 6 Hz, 3.6 W. The scanning ablation was provided in non-contact mode (1440 pulses/1 cavity), the parameters 300 mJ/per pulse, 1 Hz, mean power 0.3 W were used. Irradiated area was continuously cooled by a water spray which ensures that temperatures are well below the melting and vaporization of the enamel and dentin. A stereomicroscope and then a scanning electron microscope evaluated investigated surface. All cavities were filled by sonic activated composite resin. Four longitudinal sections were prepared after immersing in 5% methylene blue for 24?h. Microleakage was assessed quantitatively by the degree of dye penetration, data were analyzed by Fisher exact test - level of significance was set at p?<?0.05. Cavity in contact mode was smooth with a keyhole shaped prism and rod relief arrangement without a smear layer. Laser tip and tip movement have direct influence on Er: YAG laser ablation. Self-etching adhesives and sonic-activated bulk composite placement in association with the contact mode Er:YAG-lased enamel and dentin had an optimal influence on connection of enamel and resin and it could help to protect laser cavity against microleakage.

8929-9, Session 2

3D photomechanical model of tooth enamel ablation by Er-laser radiation

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The three-dimensional photomechanical model of human tooth enamel ablation is described. It takes into account: the structural peculiarities of enamel (free water in the enamel pores or cracks), beam energy spatial distribution (Gaussian) and laser radiation attenuation when radiation passes through the tissue. Dynamic change of enamel absorption coefficient during ablation is also included. We consider the water in the enamel pores is heated by laser radiation. Water boils and expands. Water begins to put pressure on the hydroxyapatite. Stresses appear in the hydroxyapatite. It is destroyed when the maximum stress exceeds ultimate strength. The modified tissue is created by this way due to incomplete removal of hydroxyapatite. As a result the enamel absorption coefficient changes during ablation. Thus the removal layer by layer occurs with different absorption coefficient. Tissue modification occurs when a unit volume of water is heated to the critical temperature. The removal of remaining hydroxyapatite also occurs due to heating by laser radiation. The remaining hydroxyapatite removal occurs when modified tissue is heated to the critical temperature which is much greater than critical temperature required for modification. The efficiency of enamel removal by single?mode YAG: Er laser radiation was investigated in experiment. We compared modeling results with experimental results. Obtained experimental dependence of the enamel removal efficiency on the energy density is in close agreement with modeling result.

8929-10, Session 3

The quest of finding the mechanism for effective root canal treatment

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For effective root canal treatment using Erbium lasers, the mechanism of action, the method and the settings are still subject of debate. To obtain a better understanding, several parameters were studied: effect of laser wavelength Er,Cr:YSGG (2.78 µm) versus Er:YAG (2.94 µm), pulse energy (10-50 mJ), pulse duration (20-300 µs), tip shape (flat or taper), position tip relative to canal (in crown or in canal), influence of gas bubbles inside canal and influence of apex (closed or open). Special high speed imaging techniques were applied to image the interaction inside an transparent root canal model with an apex chamber and ridge for debris. The canal was filled with either colored water or a mixture with carbonated water and carbon particles.

The colored water in the root canal could not be cleared as long as the apex in the model was closed independent of fiber position and shape. Total clearing was obtained fastest when the apex was closed with a soft tissue plug. The ridge filled with debris was efficiently cleaned in the presence of small gas bubbles. There were only minor differences comparing laser wavelengths, pulse duration and tip position and tip shape.

Based on the observations, the mechanism of action for effective root canal treatment is ascribed to fast moving liquid motions creating cavitation effects and microjets dissolving debris and killing bacteria which is enhanced by small gas bubbles inside the canal. Fluid motion through the apex or side canals seems essential for clearing the canal.

8929-11, Session 3

Determining optimum irradiation parameters for 1940 nm thulium fiber laser in root canal treatment

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None of the irrigants are currently used in conventional endodontic treatment has an ideal irrigant characteristics. Lasers have been used in endodontia to enchance the quality of the root canal treatment. Nd:YAG, Diode, Er, Cr:YSGG and Er:YAG lasers are the most used four lasers in endodontics. But each of them has their own limitations. A new fiber laser 1940- nm Thulium fiber Laser was chosen for this study to overcome those limitations with higher penetration and less thermal possibility of this new wavelength.

The most important problem is the high energies that are delivered to surrounding tissues can lead to irreversible thermal damage to neighboring structures. Optimum Laser power, cycling mode and type of irrigations were investigated in this study .

Temperature changes on the root surface at three regions, coronal, middle and apical parts, were recorded with a K-type thermocouple system for each experimental group and 11°C temperature increase was set to a threshold as a rule of thumb in order to prevent any irreversible thermal damage to the periodontal tissue. Root surfaces were examined microscopically in order to examine the dentinal tubules openings.

Optimum laser power range was found between 0.9 Watts and 2 watts in the first part of the experiments. While 2 Watts of laser power caused carbonization, irradiation below 0.9 Watts had no significant effect on root canal surface. In the second part of the study, two different irrigations, EDTA and NaOCl, were applied for 60 seconds while lasing and those results were compared with the control groups. All these results showed that 1940 –nm fiber is an effective tool in endodontia. The significantly best group was with irradiation of 1.5 Watts while applying 17 % EDTA during the treatment.

8929-12, Session 3

Near-infrared imaging probes for clinical transillumination and reflectance

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New technologies promising non-ionizing methods for the detection of interproximal and occlusal lesions are poised to provide clinicians

a new view into our dental anatomy. Previous in vitro studies have demonstrated near-infrared (NIR) light's utility in optical diagnosis of carious enamel tissue through transillumination and reflectance imaging geometries. Enamel is highly transparent in the NIR because light scattering decreases markedly with increasing wavelength. The use of NIR eliminates the confounding influence of stains because the organic macromolecules responsible for obscuring natural light imaging possess highly conjugated structures that do not have any absorption bands in the NIR. Three intra-oral NIR imaging probes designed for the acquisition of in vivo, real time videos using a high definition InGaAs SWIR camera and super luminescence diode lasers at 1300nm and 1600nm wavelengths. The transillumination probes provide occlusal and interproximal images using 1300nm light where water absorption is low and allows the greatest transmittance of light through enamel and dentin. The reflectance probe operates at 1600nm, where water absorption is substantially greater causing light to be trapped and extinguished in enamel. A clinical study is in progress to assess the diagnostic performance of the NIR probes and images acquired with the clinical system are displayed.

8929-13, Session 3

Automated detection of remineralization layer formation in simulated lesions with PS-OCT

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Previous in vitro and in vivo studies have demonstrated that optical coherence tomography can be used to nondestructively image the subsurface lesion structure and measure the thickness of the highly mineralized surface zone. There are structural differences between active lesions due to caries and arrested lesions, and the surface layer thickness may correlate with activity of the lesion. The purpose of my project was to develop a method that can be used to automatically detect and measure the thickness of the remineralization layer required for arresting caries progression using artificial lesion models with PS-OCT. Automated methods of analysis were used to measure the thickness of the remineralization layer and severity of demineralized bovine enamel and dentine lesions using simulated caries models that emulate demineralization in the mouth. Significant differences in lesion surface edge position from the lesions of parallel polarization and cross polarization images were detected after 4 days of remineralization. This study demonstrates that PS-OCT can automatically detect and measure thickness of the remineralization layer in simulated caries lesions.

8929-14, Session 3

Monitoring the inhibition of erosion by a CO2 laser using PS-OCT

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Since optical coherence tomography (OCT) is well suited for measuring small dimensional changes on tooth surfaces, OCT has great potential for monitoring tooth erosion. Previous studies have shown that enamel areas ablated by a carbon dioxide laser manifested lower rates of erosion compared to the non-ablated areas. The purpose of this study was to develop a model to monitor erosion in vitro that could be potentially translated to an in vivo setting. Teeth surfaces were irradiated with a carbon dioxide laser at low sub-ablative fluence to create an acid-resistant reference layer without damaging the enamel. These samples were imaged via OCT using two different erosion models; a 4.5 pH surface softened model and a pH cycling model with OJ and artificial saliva. The treated area compared with the untreated, over a course of 8 days, has shown that laser etching to prevent erosion is highly dependent on the severity of the acid challenge.

8929-15, Session 4

Change in clinical indices following laser or surgical treatment for periodontitis: a split-mouth, randomized, multi-center trial

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Data are presented from a multi-center, prospective, longitudinal, clinical trial comparing four different treatments for periodontitis, (1) the LANAP protocol utilizing a FR pulsed-Nd:YAG laser; (2) flap surgery using the Modified Widman technique (MWF); (3) traditional scaling and root planning (SRP); and (4) coronal debridement (CD). Each treatment was randomized to a different quadrant. Fifty-one (51) subjects were recruited at five centers that included both private practice and university-based investigators.

At 6-months and 12 months post-treatment the LANAP protocol and MWF yielded equivalent results based on changes in probing depths and clinical attachment levels (CAL). The major difference observed between the two procedures was that patients reported significantly greater comfort following the LANAP procedure than following the MWF ($P<0.001$).

Improvements following SRP were better than expected at 6 months and continued to improve, providing outcomes that were equivalent to both LANAP and MWF at 12 months. The improvement in the SRP quadrants suggests the hypothesis that an aspect of the LANAP protocol generated a significant, positive and unanticipated systemic (or trans-oral) effect on sub-gingival wound healing.

Analysis of changes in gingival index, recession and CAL reveals that two distinctly different post-treatment processes contribute to the interpretation of changes in CAL: (1) increases in gingival recession exposing the root surface and (2) reduction in inflammation.

8929-16, Session 4

Pulsed Nd:YAG Laser treatment for failing dental implants due to peri-implantitis

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A large percentage of dental implants experience complications, most commonly, infection leading to peri-implantitis, an inflammatory disease involving pathogen contamination. It presents with radiographic findings of crestal bone loss. At this time there appears to be no compelling evidence for an effective intervention. The LANAP protocol is a FDA cleared protocol that produces new attachment and bone regeneration

when applied to periodontally infected normal teeth. The LANAP protocol and laser dosimetry have been modified to treat peri-implantitis. Twenty clinicians who have been trained to perform LANAP and the Laser Assisted Peri-Implantitis Procedure (the LAPIP™ protocol) have volunteered 30 LAPIP case reports. The time from implant to intervention ranges from 1-9 years. Post-LAPIP radiographs range from 2-45 months. Most cases provide radiographic evidence of crestal bone regeneration around the implant and, when reported, probe depth reductions. Treating clinicians report control of the infection, reversal of bone loss and rescue of the incumbent implant. Although the success/failure rate cannot be judged from these data, any successes in this area deserve reporting and further study.

8929-17, Session 4

Effect of low level laser irradiation on implant-tissue interaction: in vivo and in vitro studies

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Laser design for surgery delivers concentrated and controllable energy to tissue which is absorbed in order to deliver biological effect of laser treatment. The present article is based on a series of in vivo and in vitro experimental studies investigating whether LLLT has the potential to enhance titanium-implant interaction. Information about LLLT effect on bone healing is fundamental to understand whether LLLT may improve implant-tissue interaction. In this study, the effect of LLLT on bone healing and growth in human tooth socket was investigated. It was found that LLLT may accelerate metabolism and/or mineralization during early bone healing..Histomorphometrical and mineral analyses showed that the irradiated implants had greater bone-to-implant contact than the controls. In the in vitro experiments, a cellular response to LLLT was studied in cell type: primary cultures of human gingival fibroblasts with special reference to attachment, proliferation, differentiation and production of transforming growth factor beta1 (TGF-beta1). The objectives of study was to develop a standardized, reproducible in vitro model for testing a GaAlAs diode laser device and to document the influence of single or multiple doses of LLLT, as a guide to defining the optimal laser dose for enhancing cell activity. While both multiple doses (1.5 and 3 J/cm2) and a single dose (3 J/cm2) enhanced cellular attachment, proliferation increased only after multiple doses. The following conclusions are drawn from the results of these studies: LLLT can promote bone mineralization and thus may be clinically beneficial in promoting bone formation in skeletal defects. It may be also used as additional treatment for accelerating implant healing in bone. LLLT can modulate the primary steps in cellular attachment and growth on titanium surfaces.

8929-18, Session 4

Effect of simvastatin versus Low Level Laser Therapy (LLLT) on bone regeneration in rabbit's tibia

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Simvastatin is a cholesterol lowering drug which proved effective on promoting bone healing. Recently low level laser Therapy (LLLT) proved its effect as a biostimulator promoting bone regeneration. This study aims to compare the effect of both Simvastatin versus low level laser on bone healing in surgically created bone defects in rabbit's tibia.

Material and methods

The study included 12 New Zealand white rabbits. Three successive 3mm defects were created in rabbits tibia first defect was left as control, second defect was filled with Simvastatin while the third defect was acted on with Low Level Laser (Diode laser 980 nm). Rabbits were sacrificed after 48 hours, 1 week and 2 weeks interval. Histopathology was conducted on the three defects

Results

The histopathologic studies showed that the bony defects treated with the Low Level Laser showed superior healing patterns and bone regeneration than those treated with Simvastatin. While the control defect showed the least healing pattern.

8929-19, Session PSun

Evaluation of the effect of a CO2 laser and fluoride on the reduction of carious lesions progression in primary teeth: an in vitro study

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This study aimed at investigating if CO2 laser irradiation(?=10.6 ?m - 11.3 J/cm2) combined with fluoridated products, enhances the CaF2 formation on enamel surface and inhibits lesion progression of demineralized primary enamel. 135 of demineralized primary enamel specimens (DES) were allocated to 9 groups (n=15): 1 - DES only 2- DES + pH cycling (control), 3- 1.23% acidulated phosphate fluoride gel (FFA), 4- 1.23% fluoride foam (FF), 5 - 5% fluoride varnish (FV), 6 - CO2 Laser (L), 7 - Laser during APF application, 8-Laser during FF application and 9-Laser during FV application. Except for the demineralized enamel group, all specimens were submitted to a 7 day pH cycling regime. The knoop hardness number (KHN) was determined by cross-sectional microhardness analysis. After treatments application, three specimens of each group had their surface examined for CaF2 formation by scanning electron microscopy (SEM). The data was analyzed by ANOVA and Student's t-test (?= 0.05). Enamel mineral loss (?S) for groups 1 to 9 were respectively: (8,676.28±1,077.46b),(12,419.54±1,050.21a),(8,156.80±1,279.90b),(8,081.32±1,019.69b),(8,820.86±1,805.99b),(8,723.45±1,167.14b),(9,003.17±796.90b),(8.229,03±961.25b),(9,023.32±1,1069b). The results showed statistically significant difference between control and all treatments groups ($p<0.05$). However there was no difference among them ($p>0.05$). SEM observations showed evidences of melting, fusion and calcium fluoride formation on enamel surface. In conclusion, laser irradiation alone or combined with fluoridated products favored the CaF2 formation on enamel surface and inhibited lesion progression of demineralized primary enamel surface. However, no synergistic effect was observed when CO2 laser irradiation and fluoridated products application and were combined.

8929-20, Session PSun

Longitudinally excited CO2 laser with short laser pulse for hard tissue drilling

Kazuyuki Uno, Hiroyuki Hayashi, Tetsuya Akitsu, Univ. of Yamanashi (Japan); Takahisa Jitsuno, Osaka Univ. (Japan)

A CO2 laser (9.2–11.4 ?m) has large absorption by water and human teeth. A short-pulse CO2 laser can produce excavation of hard tissue (dentine and enamel) without carbonization. A short-pulse CO2 laser used as a commercial dental laser should be compact and low-cost. Therefore, our objectives are the development of a short-pulse CO2 laser pumped by longitudinal pulsed discharge and the investigation of excavation characteristics of hard tissue.

The laser consisted of a 60-cm-long alumina ceramic pipe with an inner diameter of 13 mm, an optical cavity with a ZnSe output coupler with a reflectivity of 85% and a high-reflection mirror with a radius of curvature of 20 m, a pulsed power supply producing –600 V, a setup transformer, a capacitor of 1300 pF, and a spark gap. This laser device was very simple and will be low-cost, portable, maintenance-free.

The longitudinally excited CO2 laser emitted a short laser pulse with a

spike pulse width of about 100 ns and a pulse tail length of about 20 ?s like a TEA-CO2 laser, or a short laser pulse with a pulse width of 100 ns like a Q-switched CO2 laser. The laser beam was circular Gaussian beam and the full-angle beam divergence was about 2 mrad.

These laser pulses that controlled fluence were irradiated to dentine samples. The drilling characteristics were investigated by using a color laser microscope and a SEM. The short-pulse CO2 lasers produces excavation of dentine samples without carbonization.

8929-21, Session PSun

Attenuation of near-IR light through dentin at wavelengths from 1300 - 1650-nm

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

Light scattering in dental enamel decreases markedly from the UV to the near-IR and recent studies employing NIR transillumination and reflectance imaging including optical coherence tomography indicate that this wavelength region is ideally suited for imaging dental caries due to the high transparency of enamel. The opacity of dentin is an important factor in optimizing the contrast of demineralization in reflectance measurements. It also influences the contrast of occlusal lesions in transillumination. Light scattering in dentin is an order of magnitude larger than in enamel, it is highly anisotropic and has a different spectral light scattering dependence than enamel. The objective of this study was to measure the optical attenuation of near-IR light through dentin at near-IR wavelengths from 1300-1650-nm. In this study the collimated transmission of laser light through polished thin sections of dentin for various thickness from 0.1 to 0.5 mm was measured for wet samples and samples immersed in water. Beer-Lambert plots show that the attenuation coefficients range from 20 to 40 cm-1. Attenuation increased significantly with increasing wavelength and the increases were not consistent with water absorption.

8929-22, Session PSun

Integral ceramic superstructure evaluation by time domain optical coherence tomography

Cosmin Sinescu, Univ. of Medicine and Pharmacy Victor Babes Timisoara (Romania); Adrian Bradu, Univ. of Kent (United Kingdom); Florin Topala, Meda Lavinia Negrutiu, Univ. of Medicine and Pharmacy Victor Babes Timisoara (Romania); Virgil-Florin Duma, Aurel Vlaicu Univ. of Arad (Romania); Adrian Podoleanu, Univ. of Kent (United Kingdom)

Optical Coherence Tomography (OCT) is a non-invasive low coherence interferometry technique that includes several technologies (and the corresponding devices and components),, such as illumination and detection, interferometry, scanning, adaptive optics, microscopy and endoscopy. From its large area of applications, we consider in this paper a critical aspect in dentistry – to be investigated with a Time Domain (TD) OCT system.

The clinical situation of an edentulous mandible is considered; it can be solved by inserting 2 to 6 implants. On these implants a mesostructure will be manufactured and on it a superstructure is needed. This superstructure can be integral ceramic; in this case materials defects could be trapped inside the ceramic layers and those defects could lead to fractures of the entire superstructure. In this paper we demonstrate that a TD-OCT imaging system has the potential to properly evaluate the presence of the defects inside the ceramic layers and those defects can be fixed before inserting the prosthesis inside the oral cavity.

Three integral ceramic superstructures were developed by CAD/CAM technology. After the milling, the ceramic layers were applied on the core. All the three samples were evaluated by a TD-OCT system working at 1300 nm. For two of the superstructures evaluated, no defects were found in the most stressed areas. The third superstructure presented four ceramic defects in the mentioned areas. Because of those defects the superstructure may fracture. The integral ceramic prosthesis was send back to the dental laboratory to fix the problems related to the material defects found. In conclusion, TD-OCT represents a valuable method for diagnosing the ceramic defects inside the integral ceramic superstructures in order to prevent fractures at this level.

8929-23, Session PSun

Enhancing the contrast of natural occlusal lesions in OCT images with index matching agents

Hobin J. Kang, Daniel Fried, Univ. of California, San Francisco (United States)

In a previous study presented at this conference, Jones et al. 2005, we investigated the influence of several high refractive index fluids on the performance of optical coherence tomography. That study showed that these liquids could increase the effective imaging depth and lesion contrast. Other in vitro and in vivo studies have shown that OCT can be used to show whether occlusal lesions have penetrated to the dentinal-enamel junction (DEJ) and spread laterally under the enamel. The purpose of this study was to determine if high index fluids could enhance the ability of OCT to detect hidden occlusal lesions and show if these lesions have penetrated through the enamel into the underlying dentin.

8929-24, Session PSun

High contrast optical imaging methods for image guided laser ablation of dental caries lesions

Nicole LaMantia, Henry Tom, Kenneth H. Chan, Jacob C. Simon, Daniel Fried, Univ. of California, San Francisco (United States)

Our objective is to develop methods for image-guided laser ablation of dental caries. Laser based methods are well suited for automation and can be used selectively to remove dental caries to minimize the loss of healthy tissues and render the underlying enamel more resistance to acid dissolution. Aim#1 is to test the hypothesis that fluorescence and near-IR reflectance imaging methods can be used for image-guided ablation of natural non-cavitated caries lesions on occlusal surfaces. Aim#2 is to test the hypothesis that laser modification of the enamel surface does not reduce the contrast between sound and demineralized enamel in fluorescence and near-IR images. This multifaceted exploration involves first analyzing which imaging system provides suitable contrast, then pairing the system with a CO2 laser for selective decay removal and subsequent analysis of the ablation selectivity. Images will be acquired before and after laser irradiation using quantitative light fluorescence (QLF), near-IR wet and dry reflectance using 1300BP, 1460BP, 1500LP and no filter, visible light, and polarization sensitive-optical coherence tomography (PS-OCT). The sound and demineralized surfaces of twenty-five extracted human molar teeth with small non-cavitated lesions will be examined. In order for image-guided laser ablation to be feasible chemical and physical modification due to laser irradiation cannot greatly reduce the contrast between sound and demineralized dental hard tissues.

8929-25, Session PSun

Near-IR imaging of cracks in teeth

William A. Fried, Cynthia L. Darling, Kenneth H. Chan, Jacob C. Simon, Daniel Fried, Univ. of California, San Francisco (United States)

Dental enamel is highly transparent at near-IR wavelengths and several studies have shown that these wavelengths are well suited for optical transillumination for the detection and imaging of tooth decay. We hypothesize that these wavelengths are also well suited for imaging cracks in teeth. Extracted teeth with suspected cracks were imaged at several wavelengths in the near-IR from 1300-1650-nm. Extracted teeth were also examined with optical coherence tomography to confirm the existence of suspected cracks. Several teeth of volunteers were also imaged in vivo at 1300-nm to demonstrate clinical potential.

8929-26, Session PSun

Multispectral near-IR imaging of composite restorations in extracted teeth

Cooper M. Logan, Katrina U. Co, Daniel Fried, Michal Staninec, Cynthia L. Darling, 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 secondary caries develop. Dentists typically spend more time repairing and replacing composites than placing new restorations. Previous studies have shown that near-infrared imaging can be used to distinguish between sound enamel and decay due to the differences in light scattering. The purpose of this study is to use a similar approach and exploit differences in light scattering to attain high contrast between composite and tooth structure. Extracted human teeth with composites (n=15) were imaged in occlusal transillumination mode at wavelengths of 1300-nm, 1460-nm and 1550-nm using an InGaAs SWIR image sensor with a tungsten halogen light source with spectral filters. All samples were also imaged in the visible range using a high definition 3D digital microscope. Our results indicate that NIR wavelengths at 1460-nm and 1550-nm, coincident with higher water absorption, are better suited than 1300-nm for identifying composites on tooth occlusal surfaces.

8929-27, Session PSun

Near-IR imaging of demineralization under sealants

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

Previous studies have shown that near-IR reflectance and transillumination imaging can be used to acquire high contrast images of early caries lesions and composite restorative materials. The aim of the study was to determine the optimum NIR wavelengths for imaging demineralized areas under dental sealants. Fifteen natural human premolars and molars with occlusal lesions were used in this in vitro study. Images before and after application of sealants were acquired using near-IR reflectance and near-IR transillumination at wavelengths of 1300 nm, 1460 nm, and 1500 – 1650 nm. Images were also acquired using polarization sensitive optical coherence tomography for comparison. The highest contrast for near-IR reflectance was at 1460 nm and 1500 – 1650 nm. These near-IR wavelengths are coincident with higher water absorption. Contrast in the transillumination images was highest for 1300 nm. This work was supported by NIH Grants # RO1-DE 17869 and RO1-DE 14698.

8929-28, Session PSun

Indocyanine green (ICG) as a new adjuvant for the antimicrobial photodynamic therapy (aPDT) in dentistry

Joerg Meister, M. Hopp, J. Schäfers, J. Verbeek, M. Frentzen, Univ. Bonn (Germany)

Clinical surveys show a continuous increase of antimicrobial resistance related to the frequency of the administrated medication. The antimicrobial photodynamic therapy (aPDT) is an effective adjuvant to reduce the need of antibiotics in dentistry, especially in periodontics. The antimicrobial effect of light-activated photosensitizers in periodontics with blue colored derivates is demonstrated in clinical studies and case reports. Indocyanine green (ICG) as a new adjuvant shows the high potential of antiphlogistic and antimicrobial effects in combination with laser-light activation.

8930-1, Session 1

In vivo confocal (CLSM) and two-photon fluorescence microscopy (TPM) on the cornea of diabetic and non-diabetic mice

Tobias Ehmke, Friedrich-Schiller-Univ. Jena (Germany); Maria Reichard, Heike Weiss, Simone Baltrusch, Oliver Stachs, Univ. Rostock (Germany); Alexander Heisterkamp, Friedrich-Schiller-Univ. Jena (Germany)

Diabetes mellitus is one of the most widespread diseases worldwide, with neuropathy as one of the major complications. The cornea is easily accessible from the outside so that confocal laser scattering microscopy (CLSM) and two-photon microscopy (TPM) are appropriate techniques for the detection of early neurodegenerative changes in the eye. The model used in this study were B6.Cg-Tg(Thy1-YFP)16Jrs/J mice (Jackson Laboratories) where the corneal nerves show YFP-fluorescence. In this mouse-model the corneal subbasal nerve plexus of healthy and diabetic age matched thy1-YFP mice was studied under general anesthesia with both imaging methods and the nerve fiber density (NFD) was estimated with NeuronJ. Diabetes mellitus was induced in 9 weeks old mice by injection of streptozotocin (STZ). As a result of the study TPM was more sensitive to nerve detection than CLSM thus the NFD values of both techniques correlated well. With both methods it could be shown, that already after 14 days of STZ injection the NFD decreases significantly in diabetic mice (about 35%). Furthermore, the position and path of nerves within the stroma and epithelial layer can be visualized by SHG, autofluorescence and YFP excitation. Cross-sectional images can be taken and give an overview about the sample. In conclusion, confocal laser scattering microscopy and two-photon microscopy are useful tools for in-vivo observation of diabetes related nerve fiber degeneration with the additional possibility to obtain an overview of different corneal structures. This study confirms that corneal confocal microscopy can be used as a new technique for detection of diabetic neuropathy.

8930-2, Session 1

In vivo mouse corneal imaging with confocal microscopy and two-photon microscopy

Jun Ho Lee, Seong Hun Lee, Pohang Univ. of Science and Technology (Korea, Republic of); In Seok Song, Myoung Joon Kim, Asan Medical Ctr. (Korea, Republic of); Ki Hean Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

High resolution 3D imaging of the cornea is important to diagnosis corneal infection and to study corneal immune response and therapeutic effect. Confocal microscopy and two-photon microscopy (TPM) have been used for high resolution corneal imaging. Their performances have not been compared in in-vivo setting. In this study, we applied these two modalities to the imaging of mouse cornea in vivo, and compared their performance. A mouse eye holder was developed for in vivo imaging, and the corneas of control mouse eyes and suture model eyes were imaged. Balb/C mice and GFP mice were used, and a stich of suture was applied onto the cornea of these mice to induce inflammation.

In case of normal corneas of Balb/C and GFP mice, confocal microscopy visualized cellular structures in different corneal layers in either reflectance or fluorescence modes. TPM also visualized cells in individual corneal layers and collagen in the stroma based on auto- or fluorescence, and second harmonic generation (SHG) respectively. Fluorescence modes usually provided better morphological information of cells, compared to reflectance modes. In case of Balb/C suture models, TPM showed cells more clearly due to stronger autofluorescence. TPM also showed inhomogeneous distribution of collagen fibrils in the stroma based on SHG, probably due to suture and vasculature formation. TPM of GFP suture models gave similar information as that of Balb/C suture models, but with clearer morphological information of cells. Microenvironments of the cornea under inflammation were clearly visualized by using GFP mouse suture models and TPM.

8930-3, Session 1

Air-puff OCE for assessment of mouse cornea in vivo

Jiasong Li, Shang Wang, Manmohan Singh, Univ. of Houston (United States); Salavat Aglyamov, Stanislav Y. Emelianov, The Univ. of Texas at Austin (United States); Michael D. Twa, Kirill V. Larin, Univ. of Houston (United States)

The cornea provides approximately 2/3 of the optical refracting power of the eye and its shape and structure are the critical components of normal function of the eye. However, several diseases can alter biomechanical properties of the cornea and, thus, significantly reduce vision. Therefore, the use of elasticity imaging to evaluate corneal biomechanics is required for proper selection of several therapeutic procedures of the cornea, e.g. for clinicians performing refractive surgery, a practice that has motivated much of the existing research in this field. Optical Coherence Elastography (OCE) is an emerging tool that allows noninvasive assessment of tissue biomechanical properties with high lateral and axial resolutions. Here we demonstrate the use of phase-stabilized swept source optical coherence elastography (PhS-SSOCE) to assess the relaxation rate of deformation created by a focused air-pulse in tissue-mimicking gelatin phantoms of various concentrations and mouse corneas of different ages in vivo. The air-pulse and the laser beam out of the lens in the sample arm were co-focused, and the distance between the tip of the air-pulse port and the surface was kept within 300 μm during experiments. The measurement positions were chosen randomly in the relatively middle area during the phantom experiments and randomly chosen in the apex area during in vivo experiments. The results show that the relaxation rate can be quantified and is different for the varying concentrations of the gelatin phantoms and corneas of ages of the mice. The results also indicate that higher concentration of gelatin phantoms as well as older mouse corneas have faster relaxation rates. This non-contact and noninvasive measurement technique utilizes minimal force for excitation (in μm scale) of the tissue that can be potentially used to study the biomechanical properties of ocular and other sensitive tissues.

8930-4, Session 1

Evaluation of state-of-the-art imaging systems for in vivo monitoring of retinal structure in mice: current capabilities and limitations

Pengfei Zhang, Azhar Zam, Univ. of California, Davis (United States); Edward N. Pugh Jr., Robert J. Zawadzki, UC Davis Medical Ctr. (United States)

Animal models of human diseases play an important role in advancing our understanding of the cellular and molecular basis of pathogenesis, as well as the testing of new therapies. Recently several non-invasive imaging modalities including, Fundus Camera, Scanning Laser Ophthalmoscopy (SLO) and Optical Coherence Tomography (OCT) have been successfully applied to monitor structural changes in the retinas

of the living animals, allowing experiments where an individual animal is followed over the time course of disease progression or therapeutic intervention. Here we evaluate the capabilities and limitations of several imaging modalities including fundus camera (Micron III), SLO and OCT for visualization of specific structures in the mouse eye. Example images acquired from wild type mice and mouse models of retinal disease will be presented. Future directions of development for these instruments and potential advantages of multi-modal imaging systems will be presented as well.

8930-5, Session 1

In vivo recording of intrinsic optical signals in light-stimulated rat retina with a combined functional OCT and ERG system

Bingyao Tan, Man Chun A. Tam, Kirsten Carter, Ameneh Boroomand, Alexander Wong, Kostadinka Bizheva, Univ. of Waterloo (Canada)

Visually evoked intrinsic optical signals (IOS) were measured in-vivo from individual layers of the rat retina with a high resolution functional OCT, combined with a commercial ERG system (fOCT+ERG). The fOCT system operates in the 1060nm spectral range and provides 3.2μm axial resolution and acquisition rate of 92,000 A-scans/s. The fOCT imaging probe was integrated with a custom built multicolor visual stimulator from which light is projected onto the retina to form a uniformly illuminated spot with precisely controlled intensity and duration of the visual stimulus. Both dark (no flash) and single flash fOCT and ERG recordings were collected from dark adapted rat retinas. The fOCT images were aligned, denoised and segmented automatically and the IOS were computed for each retinal layer. Both positive and negative IOS were measured from different retinal layers, which showed very good time correlation with the simultaneously recorded ERG traces.

8930-6, Session 1

In vivo optical coherence tomography of intracellular pigment migration in retinal pigment epithelium

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Optical coherence tomography (OCT) may revolutionize fundamental investigation and clinical management of age-related macular degeneration and other eye diseases. However, quantitative OCT interpretation is hampered due to uncertain sub-cellular correlates of reflectivity in the retinal pigment epithelium (RPE) and photoreceptor. The purpose of this study was to test OCT correlates in the RPE. A high resolution spectral-domain OCT was constructed to achieve dynamic imaging of frog eyes, in which light-driven translocation of RPE melanosomes were known to occur within the RPE cell body and apical processes. In dark-adapted frog eyes, in vivo OCT imaging consistently revealed a bright hyper-reflective band at the RPE. After the frog eye was exposed to visible light illumination or in light-adapted frog eyes, the reflectivity of RPE band was decreased significantly. In contrast, the reflectivity of bands attributed to inner segment ellipsoid and OLM was enhanced. The observed OCT dynamics showed a tight correlation with light driven melanosome translocation in the RPE cell body and apical processes. Histological examination confirmed the light induced melanosome redistribution in dark- and light-adapted frog eyes. This is the first demonstration of in vivo observation of melanosome migration in frog retina/RPE using a custom-designed functional OCT. In vivo OCT recording of melanosome migration in the retina/RPE can not only foster better understanding of the light regulatory processes in the retina/RPE complex, but also opens

8930-7, Session 2

Five-dimensional analysis of multi-contrast Jones matrix tomography of posterior eye

Udaya Bhaskar, Univ. of Tsukuba (Japan) and Indian Institute of Technology (India); Young-Joo Hong, Univ. of Tsukuba (Japan); Masahiro Miura, Tokyo Medical Univ. (Japan); Yoshiaki Yasuno, Univ. of Tsukuba (Japan)

Multi-contrast Jones matrix tomography (MC-JMT) provides a structural OCT, Doppler OCT and polarization sensitive OCT. For effective tissue discrimination by MC-JMT, a proper image processing before segmentation process is required. This presentation presents a five-dimensional analysis of MC-JMT images. In this analysis, all pixels of MC-JMT are projected into five dimensional features space, where the features are depth and transversal spatial positions, logarithmic OCT intensity, squared power of Doppler shift, and degree of polarization uniformity. By clustering the pixels in this five dimensional feature space, spatially localized cluster of pixels, so called superpixels, are obtained. The MC-JMT pixels in a same superpixels are then averaged. Finally, a compact MC-JMT image is obtained. This compact image is compact in the number of effective pixels, and has less noise because of the averaging. In addition, since the clustering of the superpixel was done by using both the spatial and optical features, the shape of the superpixels tends to preserve the structural property of the sample. And hence, despite of the compact ness, the superpixel image preserves the sample structure. By further processing the superpixel image, a stable and robust segmentation of ocular posterior tissue is demonstrated.

8930-8, Session 2

Retinal tracking polarization sensitive optical coherence tomography of the diseased eye

Mitsuro Sugita, Medizinische Univ. Wien (Austria) and Canon Inc. (Japan); Stefan Zotter, Michael Pircher, Philipp Roberts, Medizinische Univ. Wien (Austria); Tomoyuki Makihira, Canon Inc. (Japan); Kenichi Saito, Canon U.S.A., Inc. (Japan); Nobuhiro Tomatsu, Makoto Sato, Canon Inc. (Japan); Ursula Schmidt-Erfurth, Christoph K. Hitzenberger, Medizinische Univ. Wien (Austria)

Polarization sensitive optical coherence tomography (PS-OCT) is a powerful tool for differentiating retinal layers with polarization preserving, birefringent, and depolarizing characteristics. In real clinical situations of ophthalmologic studies, involuntary eye motions and poor fixation associated with eye diseases cause significant motion artifacts in the OCT images. In order to achieve high quality and more robust imaging in real clinical studies, we developed a new PS-OCT system with an integrated retinal tracker. 3D datasets of 1024 ? 250 raster scan were recorded with an acquisition speed of 70 kA-scan/sec. (4.5 sec/volume). M-mode B-scans were also acquired and used for frame averaging. The system was used to image eyes of glaucoma patients and patients with AMD. Averaged retardation images from glaucoma eyes showed clearer build-up of retardation in the retinal nerve fiber layer. PS-OCT images from eyes with wet AMD demonstrated loss of the retinal pigment epithelium (RPE) and its replacement by birefringent scar tissue of varying collagen fiber orientation. These exemplary results demonstrate the capability of our tracking PS-OCT system in real clinical situations and the benefit of the high-quality images by frame averaging with stabilized B-scans. Further results acquired from different patient eyes will be also presented.

8930-9, Session 2

Imaging pigmented structures in the rat eye using polarization sensitive optical coherence tomography

Bernhard Baumann, Sabine Rauscher, Medizinische Univ. Wien (Austria); Martin Glösmann, Veterinärmedizinische Univ. Wien (Austria); Erich Götzinger, Micheal Pircher, Stefan Zotter, Marion Gröger, Wolfgang Trasischker, Teresa Torzicky, Christoph K. Hitzenberger, Medizinische Univ. Wien (Austria)

We present polarization sensitive optical coherence tomography (PS-OCT) for imaging pigmented tissues in the rat eye. Ocular structures containing melanin such as the retinal pigment epithelium (RPE) and the choroid are playing key roles in age-related macular degeneration (AMD). PS-OCT is a functional extension of OCT and enables the measurement of polarizing properties such as birefringence and depolarization. In this presentation, in vivo high speed spectral domain PS-OCT imaging is shown in the eyes of both pigmented and albino rats. Similar to the appearance in human eye, depolarization was observed in the RPE/choroid complex of pigmented rats. The histological analysis of both albino and non-albino rat eyes confirmed that only structures containing melanin granules revealed depolarization in PS-OCT. The capability of PS-OCT to image melanin pigments in the eye based on their intrinsic polarization properties paves the path to the development of non-invasive tools for detecting and measuring irregular pigmentation which may improve early diagnosis of vision threatening diseases such as AMD.

8930-57, Session Key

Corneal refractive surgery: is intracorneal the way to go and what are the needs for technology? *(Keynote Presentation)*

Jesper O. Hjortdal M.D., Aarhus Univ. (Denmark)

No Abstract Available

8930-10, Session 3

Eye vision system using programmable micro-optics and micro-electronics

Nabeel A. Riza, Muhammad Junaid Amin, Univ. College Cork (Ireland); Mehdi N. Riza, Presentation Brothers College (Ireland)

Eye vision correction measurement is traditionally done using bulky opto-mechanical systems called phoropters that commonly have large moving parts with minimal handheld portability. The desire to use electrically programmable optical lens devices to replace spectacles (contacts or glasses) for every-day use has been around since the 1970s. Starting in the mid-1980s, the General Electric Corporate Research & Development Center (GE-CRD) fabricated novel electronically controlled Liquid Crystal (LC) lens devices, including for eye correction measurement applications. Use of liquid-based lenses using mechanical pressure to change lens focal length has also been proposed for phoropters in 1995 and also more recently. In addition, apart from using an electronic LC lens in a phoropter to get new eyewear refractive readings, two dimensional (2-D) optical spatial light modulator devices (via LC and micromachined or MEMS) devices have be proposed for color blindness tests as well as to provide the capability to perform eye strain relief and eye muscle exercises. This paper proposes an eye vision system that combines the use of advanced micro-optic and microelectronic technologies that includes micro-optic liquid lenses, MEMS)/LC/Laser Scanner Pico-projectors and smart optics, and RF wireless electronics to deliver a portable light weight system that can measure eye refractive powers, conduct color-blindness tests, and also implement eye strain relief and eye muscle exercises via time sequenced imaging. The paper starts with the basic design of the system and describes first stage system experimental results for vision spherical lens refractive error correction.

8930-11, Session 3

High temporal resolution ocular aberrometry with pupil tracking

Jessica Jarosz, ONERA (France) and Quantel Medical (France); Serge C. Meimon, Jean-Marc Conan, ONERA (France); Michel Paques, CHNO des Quinze-Vingts (France)

While state-of-the-art adaptive optics retinal imaging research systems yield unprecedented resolution bordering on the diffraction limit, further research is to be achieved in therapeutic adaptive optics systems, in particular for laser photocoagulation. Major problems encountered with today's photocoagulation systems are due to dynamic ocular aberrations, especially eye movements and micro-accommodations of the crystalline lens.

Although most ophthalmic adaptive optics systems are directly inspired from astronomy, more cost effective and robust designs could probably be derived from a thorough knowledge of ocular aberrations. Ocular aberration statistics are actually very different from what is found in astronomy, as they are neither temporally nor statistically (across the population) stationary. Unfortunately, high frequency temporal statistical behavior of ocular aberrations remains poorly characterized. Besides, although it is generally agreed that dynamic aberrations originate mostly from micro-accommodations, ocular movements and the tear film, the balance between these contributors is still controversial.

We present an original custom-built Shack-Hartmann aberrometer with pupil tracking. Our set-up features high temporal frequency (higher than 100Hz) and high spatial frequency (sampling around 20x20 lenslets). We carried out a series of measurements over a population of 50 subjects. First, calculation of the phase, with and without correction from pupil shifts, enabled us to conclude on the importance of ocular movements in the dynamics of aberrations. Second, this measurement campaign provided us with precious and relevant data which expand existing statistical ocular dynamic models. Finally, we draw first conclusions on the design of adaptive optics systems for laser photocoagulation.

8930-12, Session 3

Simple handheld pupillometer for chromatic Flicker studies

Mario Bernabei, Univ. degli Studi di Modena e Reggio Emilia (Italy); Roberto Tinarelli, Lorenzo Peretto, Univ. degli Studi di Bologna (Italy); Luigi Rovati, Univ. degli Studi di Modena e Reggio Emilia (Italy)

We have developed a portable pupillometer capable of precise measurements of pupil diameter during chromatic flicker stimulations. The handheld measuring system records the near-infrared image of the pupil at the rate of 30 fps and simultaneously stimulates the eye using a diffused flicker light. Intensity, frequency and chromatic coordinates of the stimulus can be easily adjusted using a user-friendly graphical interface. Thanks to a precise chromatic monitoring of the stimulus close to the plane of the eye, equiluminant and/or photopically matched conditions can be easily achieved. Even if the size of the instrument is extremely compact, thanks to a single plano-convex lens a stable fixation image is presented to the patients. The pupil images is acquired during the flickering stimuli with frequency in a range of 1–20 Hz and offline processed exploiting the Sobel filter algorithm to get the pupil edge. Pupil diameter/area is obtained by fitting this edge by a circular shape. Low measurement uncertainty and repeatability are the most important

characteristics of the developed pupillometer. This paper describes the instrument optical setup, front-end electronics and data processing. Preliminary experiments performed in our laboratory shows a constant pupillary constriction in response to a flickering stimulus. The amplitude and duration of this response is related to color and temporal frequency of the stimulus.

8930-13, Session 3

High frequency pupillometry

Serge C. Meimon, ONERA (France); Jessica Jarosz, ONERA (France) and Quantel Medical (France)

Our eyes are continuously in motion even at rest. Whether fixating or following a moving target, eye movements are essential to maintain optimal vision. However, in high resolution imaging systems as adaptive optics retinal imagers, these fixational eye movements result in performance degradation or in cost/complexity increase, as they induce a movement of the entrance optical pupil of the system. The knowledge of the 3D movement of the eye pupil is a key to design more accurate and cost effective adaptive optics systems. Indeed, the third dimension, corresponding to the displacement of the pupil along the optical axis, induces a conjugation error between the location of the eye aberrations (cornea, cristal lens) and the deformable mirror.

Fixational eye movements combine tremors along with drifts and microsaccades [Martinez-2004]. Recently, measurements using ellipse fitting report a 2.5 micron precision at 63Hz [Roig2011]. In a first study focusing on 2D movements of the pupil, we have been able to obtain a precision below 2 microns at a 483Hz frequency, unprecedented to our knowledge.

The have also conducted two other studies, aiming at measuring 3D pupil movements, with and without a chin rest. Results of measurements over more than 100 subjects are presented.

8930-14, Session 3

Novel technique: a pupillometer-based objective chromatic perimetry

Ygal Rotenstreich, Alon Skaat, Ifat Sher, Tel Aviv Univ. (Israel); Andru Kolker, George Washington Univ. (United States); Elkana Rosenfeld, Shlomo Melamed, Michael Belkin, Tel Aviv Univ. (Israel)

PURPOSE: To evaluate a novel objective perimetry using multifocal chromatic pupil light reflex in normal participants and patients with retinitis pigmentosa (RP) or glaucoma.

METHODS: A computerized infrared video pupillometer was used to record changes in pupil diameter in response to short- and long-wavelength stimuli (peak 485 nm and 620 nm, respectively) at light intensities of 15-100 cd/m2 at thirteen different points of the visual field. The RP study included 16 eyes of 8 patients and 18 eyes of 11 normal participants. The glaucoma study included 22 eyes of 11 patients and 38 eyes of 19 normal participants.

RESULTS: Significantly reduced pupillary responses (PR) were obtained in RP patients in nearly all perimetric locations in response to short-wavelength stimulus. RP patients demonstrated significantly reduced PR mostly in peripheral locations in response to long-wavelength stimulus. In a cone-rod dystrophy patient, the PR to both long- and short-wavelength stimuli was significantly lower in the scotoma area identified by the dark-adapted chromatic Goldmann perimetry. In all patients, minimal PR was recorded in areas that were non-detected in the chromatic Goldmann. Glaucoma patients demonstrated significantly reduced PR in response to high intensity short- and long-wavelengths in all perimetric locations. PR recordings correlated with Humphrey-based perimetry in majority of VF locations.

CONCLUSIONS: This study demonstrates the feasibility of using pupillometer-based chromatic perimetry for objectively assessing visual field defects and retinal function in patients with retinal dystrophies and glaucoma. This method may be used to distinguish between the damaged rod/cone/ganglion cells underlying the VF defect.

8930-15, Session 4

Complete 360° circumferential SSOCT gonioscopy of the iridocorneal angle

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The ocular iridocorneal angle is generally an optically inaccessible area when viewed directly through the cornea due to the high angle of incidence required and the large index of refraction difference between air and cornea (na = 1.000 and nc = 1.376) resulting in total internal reflection. Gonioscopy allows for viewing of the angle by removing the air-cornea interface through the use of a special contact lens on the eye and is used clinically to visualize the angle directly but only en face. Optical coherence tomography (OCT) has been used to image the angle and deeper structures via an external approach. Typically, this imaging technique is performed by utilizing a conventional anterior segment OCT scanning system. However, instead of imaging the apex of the cornea, either the scanner or the subject is tilted such that the corneoscleral limbus is orthogonal to the optical axis of the scanner requiring multiple volumes to obtain complete coverage of the ocular angle. We developed a novel gonioscopic OCT (GOCT) system that images the entire ocular angle within a single volume via an “internal” approach through the use of a custom radially symmetric gonioscopic contact lens. We present, to our knowledge, the first complete 360° circumferential volume of the iridocorneal angle from a direct, internal approach.

8930-16, Session 4

Portable, low-priced retinal imager for eye disease screening

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The objective of this project was to develop and demonstrate a portable, low-priced, easy to use non-mydriatic retinal camera for eye disease screening in underserved and rural locations. Existing portable retinal imagers do not meet the requirements of a low-cost camera with sufficient technical capabilities (field of view, image quality, portability, battery power, and ease-of-use) to be distributed widely to low volume clinics, such as the offices of single primary care physicians serving rural communities or economically stressed healthcare facilities. Our innovative approach for i-RxCam™ is based primarily on a significant departure from current generations of desktop and hand-held commercial retinal cameras as well as those under development. Our innovations are: 1) Exclusive use of off-the-shelf components; 2) Integration of imaging device into low-cost, high utility camera mount and chin rest; 3) Unique optical and illumination designed for small form factor; and 4) Exploitation of autofocus technology built into present digital SLR recreational cameras; 5) Integration of a polarization technique to avoid the corneal reflex. In a prospective study, 41 out of 44 diabetics were imaged successfully. No imaging was attempted on three of the subjects due to noticeably small pupils (less than 2mm). The images were of sufficient quality to detect abnormalities related to diabetic retinopathy, such as microaneurysms and exudates. These images were compared with ones taken with a Canon CR-1 Mark II camera, and no cases identified as having DR by expert retinal graders were missed by the i-RxCam™.

8930-17, Session 4

Non-mydriatic, wide field, fundus video camera

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We propose a method for a non-mydriatic fundus video camera that is capable of acquiring wide field color videos of the fundus of the human eye. In our method the aperture is bisected in two half circles to suppress the 1st and 2nd Purkinje reflections. One half is used for observation. The light bundle for illumination crosses the other half of the pupil with its waist and diverges towards the fundus to a light bar. The 3rd and 4th Purkinje reflections are focused at two unused areas, which are faced at the periphery of the image plane. The gap between them is free of reflections and contains the stripe-shaped field of view. Compared to common used ring illumination methods we reach the same viewing angle in one direction, but can theoretically arbitrary expand the viewing angle in the orthogonal direction. This yields a very wide stripe-shaped field of view even at small pupil sizes. We designed a demonstrator and successfully could acquire color videos of the fundus with 68° x 18° field of view at 4 frames per second. We tracked and compensated eye movements by digital post processing. We could observe a massive change of blood vessels in the region of the papilla when a subject slightly pressed at his lid. Simultaneously the papilla became brighter. We showed that the camera can also be used to generate one high quality image by averaging several video frames.

8930-18, Session 4

Handheld simultaneous color SLO/OCT instrument

Francesco LaRocca, Derek Nankivil, Sina Farsiu, Joseph A. Izatt, Duke Univ. (United States)

Scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT) are widely used retinal imaging modalities that can assist in the diagnosis of retinal pathologies. SLO and OCT provide complementary image information at different time scales and when combined, rapidly acquired 2-D en-face SLO images may be used to register volumetric OCT B-scans to correct for patient motion within an OCT volume. In addition to structural information, SLOs can provide color information of the retina by utilizing a single white or separate red, green, and blue light sources and detecting via red, green, and blue channels. Previous color-SLO imaging techniques utilized three color lasers for illumination using tabletop SLO systems. We present the first demonstration of a handheld color-SLO/OCT system; this is the first handheld color-SLO and the first color-SLO with OCT. A filtered supercontinuum source and broadband superluminescent diode were used for color-SLO and OCT imaging, respectively, with a combined power under the ANSI limit. Collected SLO light was transferred via multimode fiber to a separate module to split the light into three color channels. Images from the three channels were registered, averaged and combined to create color images with the superior contrast and axial sectioning capability of SLOs as compared to fundus photography.

8930-56, Session 4

The Uppsala Contrast Sensitivity Test (UCST): a fast strategy for clinical assessment of contrast sensitivity

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SWEDEN

Routine clinical measurement of spectral contrast sensitivity is hampered by current methods. Presently, contrast sensitivity has to be recorded iteratively, with separate measurements for each spatial frequency. We are developing a system that allows instantaneous measurement of spectral contrast sensitivity.

The UCST system consists of custom designed software running on an iPad connected to a calibrated TFT-monitor. Twenty healthy subjects were consecutively randomized to have their spectral contrast sensitivity measured with the UCST strategy or with a Vistech VCTS 6500 chart. The examination time and the spectral contrast sensitivity, respectively, were recorded for each eye in each subject.

The Vistech strategy resulted in a more extended mean examination time (CI-Vistech:?(0.95) = 87 ±27 s, d.f. = 9) than the UCST strategy (CI-UCST:?(0.95) = 13 ±4 s, d.f. = 9), and the estimated mean difference between the two strategies indicated a difference in examination time (CI-difference:?(0.95) = [47;106] s, d.f. = 18).

The overall contrast sensitivity for each group was estimated as the contrast sensitivities for the spatial frequencies sampled, integrated over the spatial frequency band sampled. The Vistech strategy resulted in a higher estimated mean overall contrast sensitivity (CI-Vistech:?(0.95) = 116±24 log rel.•log [c.•deg-1], d.f. = 9) than the UCST strategy (CI-UCST:?(0.95) = 74±14 log rel.•log [c.•deg-1], d.f. = 9), and the estimated mean difference between the two strategies indicated a difference in overall contrast sensitivity (CI-difference:?(0.95) = [15;68] log rel.•log [c.•deg-1]), d.f. = 18).

It is concluded that the UCST strategy measures spectral contrast sensitivity on the order of 7 times faster than Vistech strategy. The slightly lower overall contrast sensitivity recorded for the UCST strategy appeared to be due to a limitation in dynamic range that can be overcome with improved design.

8930-19, Session 5

Novel publicly available denoising algorithm improves the performance of automated ophthalmic segmentation algorithms

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Accurate quantification of corneal and retinal layers' thicknesses in SD-OCT images of human eyes is crucial for the study of many ophthalmic and neurologic diseases. Segmenting these layers manually is inherently subjective and time-consuming. However, successful automated segmentation of anatomic and pathologic features on ocular SD-OCT scans requires high signal-to-noise ratio (SNR) images. Several novel denoising algorithms have been shown to better improve the SNR of SD-OCT images in comparison with classic methods. However, for ophthalmic SD-OCT applications, few of these novel denoising algorithms have proven superior to classic denoising in effectively improving the accuracy of segmented layer boundaries. In this work, we show that our novel publically available denoising algorithm (Fang et al. IEEE TMI, In Press 2013), which we termed sparsity based simultaneous denoising and interpolation (SBSDI), significantly improves the performance of corneal and retinal layer segmentation algorithms as compared to classic and the state-of-the-art denoising methods. First, we characterize the lamellar cut in endothelial keratoplasty corneal transplantation, by automatically identifying the intrastromal lamellar dissection and the endothelial surfaces on the low-quality SD-OCT images of precut donor tissue. Second, we segment the weakly visible choroid/sclera junction in unaveraged retinal SD-OCT images. The preliminary results demonstrate the efficacy of our novel denoising algorithm for improving the accuracy of the automated segmentation methods. Especially in corneal layer segmentation applications, quantitative results showed that our automatic segmentation better

matches the gold standard of manual segmentation, when utilizing SBSDI method.

8930-20, Session 5

Sub-cellular segmentation of adaptive optics - optical coherence tomography images

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Widespread clinical application of optical coherence tomography (OCT), and the attendant need for computer-assisted diagnostic tools, have driven the development of a diverse set of approaches for segmenting 2-D and 3-D tomographic images of the retina. The goal of all of these approaches has been the segmentation and identification of retinal layers, the thickness and uniformity of which have become key criteria in the diagnosis and evaluation of retinal disease. None of the existing methods, however, have sought to segment cellular or sub-cellular structures, as the instruments employed in clinics rarely possess sufficient resolution (axial or lateral) to resolve such structures. Adaptive optics (AO) OCT, in contrast, provides sufficient resolution for 3-D resolution of cells--and sub-cellular structures--in the retina. Volumetric AO-OCT images may contain hundreds or thousands of any given class of such structures, and in order to perform thorough quantitative analyses of these images, automated techniques are needed for identifying and segmenting those structures. Here we present such a technique for automated segmentation of microscopic structures of the outer retina, the outer segments (OSs) of the photoreceptors in particular.

8930-21, Session 5

Automatic segmentation of nine layers in SD-OCT images of the mouse retina with and without pathology

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Accurate quantification of retinal layers' thicknesses in SD-OCT images of murine eyes is crucial for the study of many ophthalmic and neurologic diseases in humans. However, segmenting these layers manually is inherently subjective and time-consuming. While many automated algorithms for segmenting retinal layers in human eyes have been developed [1], few address the segmentation of murine eyes. Previous attempts to address this problem [2] were limited in application as the test images were preselected considering limiting criteria including: 1) The test images were chosen from normal eyes or diseased eyes in which no retinal layer was completely missing. 2) The test images were limited to the central slices of the volumes where all retinal layers were clearly visible, eliminating images from the periphery of the retinal volumes where retinal layers had lower quality and images of the optic nerve where several layers disappear. Based on our generalized graph theory and dynamic programming (GTDP) framework, we present a novel segmentation methodology which is capable of accurately segmenting the retinal layers of normal and diseased eyes in images from all sections of the retina, including periphery and the nerve, with missing layers and significant pathology. The qualitative results demonstrate the extensibility of our GTDP framework to accurately segment retinal layers of murine eyes in SD-OCT images, even in the presence of unexpected anatomic and pathologic features. Quantitative results show that our automatic segmentation closely matches the gold standard of manual segmentation on images from normal mice and mice models of retinal degeneration.

8930-22, Session 5

Segmentation method for in vivo meibomian gland OCT image

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We report the segmentation method for the meibomian gland (MG) OCT images under a human eyelid. Quantified volume information of the meibomian gland is helpful to diagnose whether the MGD or the lacrimal gland dysfunction. The everted upper eyelid of a subject was imaged using a swept-source OCT (SS-OCT) system to obtain the tomograms of the MG. The SS-OCT system had 1310nm wavelength swept source for deeper tissue imaging. The upper eyelid of a subject was examined by a near infrared (NIR) meibography to confirm the OCT imaging range. The SS-OCT scan range of the upper eyelid was 5 mm x 2 mm. We developed image processing protocol to extract the MG area. The first step of the MG segmentation procedure is aligning and flattening process of the obtained OCT images. It is helpful to reduce the axial eyelid vibration from the 3D imaging process. The second step is conjunctiva removal and thresholding process. The segmented MG area was converted into a binary image. We could calculate the pixel number of the MG area from the segmented OCT image. The proposed method can be applied into quantifying volume information of the MGs and to lead to index of the meibomian gland activity.

8930-23, Session 6

Challenging evolution for a different design of the human visual system

Pier Giorgio Gobbi, Scientific Institute Hospital San Raffaele (Italy)

The overall design of the human visual system seems affected by several flaws, among which aberrations (monochromatic and chromatic), limited dynamic range, limited spatial and temporal resolution. Why did it evolve this way? Could it be artificially optimized in some respect?

The performance of the human visual system is analysed by means of the CAGE-Barten eye model, which is a neuro-physical model able to give quantitative estimates of optical and visual performances of the human eye, in good agreement with experimental measurements (P.G. Gobbi, Optical and visual performance of the human eye, SPIE Press 2013). In particular, the analysis is done in the contrast-frequency plane, where the model defines the entire region of perception, outlining basic parameters as maximum visual acuity and maximum contrast sensitivity.

Optical performances would marginally benefit by correction of spherical aberration, much more through the nulling of chromatic aberration. However, the greatest limitation on visual performance is set by the level of neural noise that is developed along the complex neural mesh connecting retinal photoreceptors to visual cortex, where parallel processing of visual information is performed. Without aberrations and neural noise, visual acuity could virtually rise from 1.6 to 3.7, and contrast sensitivity could improve by two orders of magnitude. However, a number of processing tasks (among the others: edge detection, color coding, discrimination of orientation, size and movement) would be lost or substantially limited and slowed down.

8930-24, Session 6

Finite element study on the effects of GRIN order on the accommodative response of the human crystalline lens

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The gradient refractive-index (GRIN) inside the crystalline lens has been described using a number of functions in the literature. One of the most widely used functions for this purpose is the polynomial. Changing the order of the GRIN polynomials alters the relative refractive index profile across (radially) and along (axially) the lens. In this paper, numerical methods are used to investigate the effects of varying the GRIN polynomial order on the accommodative response of the lens; in particular, accommodative amplitude and aberrations. Our results show that while the GRIN order can alter the lens aberrations, it does not have a significant influence on the accommodation amplitude.

8930-25, Session 6

Accuracy evaluation of scleral lens thickness and radius of curvature using high-resolution SD- and SS-OCT

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Anterior segment optical coherence tomography (AS-OCT) is capable of measuring morphometric properties of the cornea such as pachymetry while at the same time delivering high-resolution cross-sectional images of corneal tissue, revealing a variety of pathological changes. The purpose of this study was to evaluate the accuracy of high resolution spectral domain (SD) and swept source (SS) optical coherence tomography (OCT) systems as well as the Oculus Pentacam™ when t used for central thickness measurements. Fourteen scleral lenses were used as a phantom target. Physical thickness measurements were obtained from 7 marked positions of each lens with a thickness gauge and subsequently the lenses were imaged with an Oculus Pentacam, a SD-OCT and a SS-CT. No significant difference was observed between the thickness measurements with the gauge and either of the two OCT systems ($p>0.05$). These findings indicate that once calibrated properly, the SD- and SS-OCT systems measure the lens thickness parameters with notable accuracy.

8930-26, Session 6

Correlation of glaucoma severity with OCT-derived reference-free RNFL attenuation coefficients

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Attenuation coefficients, which may be estimated from optical coherence tomography (OCT) data, provide a way to assess the tissue's health. In the context of glaucoma diagnosis and monitoring, assessment of the retinal nerve fiber layer (RNFL) is of primary concern. Previously, a method to estimate the RNFL attenuation coefficient, based on normalization on the retinal pigment epithelium, was introduced. More recently, a reference-free method was presented that enables the calculation of depth-resolved attenuation coefficients.

We present an evaluation of the correlation between severity of glaucoma and the RNFL attenuation coefficient, as determined by the reference-free method. Peripapillary scans of 10 eyes of normal, early glaucoma, moderate glaucoma and advanced glaucoma were acquired on a Spectralis (Heidelberg Engineering, Germany) OCT system. Attenuation coefficients were determined from the OCT scans and averaged over the RNFL. The built-in software algorithms of the instrument were used to segment the RNFL.

A Jonckheere-Terpstra test was performed on the data, indicating a highly significant trend ($p<10^{-5}$) of decreasing RNFL attenuation coefficients with increasing severity. The RNFL atttenuation coefficient was significantly correlated with mean deviation ($p<10^{-4}$). Mann-Whitney U-tests were performed between all groups and showed that RNFL attenuation coefficients of glaucomatous eyes were significantly smaller ($p<=0.001$) than those of normal eyes.

The attenuation coefficient of the RNFL decreases with increasing severity of glaucoma. Attenuation coefficients are most strongly affected in the early stages of glaucoma. No significant differences between glaucoma stages could be found due to the small number of eyes per group.

8930-27, Session 6

Retinal gain evaluation

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Purpose. To evaluate the retinal gain from schematic models of the human eye. The retinal gain (RG) is an amplification factor given by the ratio of retinal irradiance to corneal irradiance for an optical beam propagating into the eye. It is of relevance for retinal safety against overexposure levels.

Methods. Four finite eye models were considered: the Kooijman (K) model, the Navarro-Santamaria-Bescòs (NSB) model, the Liou-Brennan (LB) model, and the chromatic aspherical Gullstrand exact (CAGE) model. Their aberration function was calculated through ray-tracing, and the point spread function evaluated by means of physical optics propagation (Fresnel approximation). For the last three models, the retinal gain could be evaluated not only for monochromatic radiation, but also for broadband radiation.

Results. Due solely to diffraction, RG would grow up with the fourth power of the pupil size. However, aberrations concomitantly degrade optical performance and as a result RG tends to stabilize at a constant level, for large pupil sizes. In monochromatic illumination, the behavior is accompanied by damped oscillations around the plateau, which are due the combined action of diffraction and spherical aberration on a coherent optical field. At the plane of minimum blur, the maximum values of RG attained by the different models are: 13.7×10^5 (K), 15.6×10^5 (NSB) and 17.5×10^5, while the LB model provides the much larger value of 34.1×10^5. With broadband radiation, oscillations disappear due to the smoothing given by the incoherent superposition of various monochromatic profiles; the plateau values amount to: 8.9×10^5 (NSB), 9.6×10^5 (CAGE), and 17.5×10^5 (LB).

Discussion. The LB model provides RG values twice as large than for the other three models, due to its underestimate of ocular spherical aberration. The K, NSB and CAGE models are grossly aligned to each other, but their RG estimates are 7 to 9 times larger than the value of 2×10^5, which is assumed by ocular safety standards for optical radiation. This result implies that the safety margin included in all exposure standards is vanished or greatly reduced.

8930-28, Session 7

Label-free SHG imaging and spectral FLIM of corneas using a sub-15 fs laser microscope

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Alterations to the metabolism or to the structural organization of the collagen fibrils occur in several corneal and systemic pathologies. We aim to assess these modifications by using spectral FLIM on corneal tissues, imaging NADH and flavin autofluorescence, and SHG of collagen fibrils.

We resorted to a sub-15fs NIR laser with a large 100nm spectral bandwidth combined with a 16 channels PMT detector. This allows the simultaneous analysis of both metabolic co-factors, as well as collagen SHG. Fresh porcine corneas, obtained in the local slaughterhouse, were used. In addition, human cornea was investigated. Aluminium foil as well as special mirrors were placed on top of the sample to increase SHG signal.

With our experimental setup it was possible to image corneal epithelial layer and to discriminate between NADH and flavins autofluorescence. In both spectral ranges two lifetime components were detected. For NADH spectral range, lifetimes of 1ns (76%) and 3.5ns (24%) were detected, whereas in the flavin spectral range, lifetimes of 0.3ns (67%) and of 1.8ns (33%) were observed.

In the corneal stroma it was possible to detect SHG from collagen fibrils. In SHG spectral range a very fast decay (0.1ns) was observed.

For the first time, one can image simultaneously NADH autofluorescence, flavin autofluorescence, and SHG of collagen fibrils, thus a full characterization of the corneal status is possible. This can be of great importance towards a better understanding of corneal and systemic dystrophies.

8930-29, Session 7

Biometry of the ciliary muscle during dynamic accommodation assessed with OCT

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While it is accepted that changes in size and mechanical and optical properties of the crystalline lens are central to presbyopia, very little is known about the ciliary muscle and how it may or it may not contribute to the loss of accommodation with age. Although the anatomy of the ciliary muscle is generally understood, the exact structural changes with age and during accommodation are still unclear. TD-OCT and SD-OCT have been used to detect thickness changes in the ciliary muscle (CM) at different age and accommodative states with low resolution and at a slow speed. Dynamic imaging and biometry of the CM requires high speed and high resolution to detect the subtle changes of the CM structure during accommodation. Accurate biometry of the CM also requires the OCT images to be corrected for measurement errors introduced by the optical distortion due to refraction of the probe beam at the different ocular interfaces, for different curvature of the scleral wall across subjects and for eye movements occurring while imaging dynamic accommodation. In this study, we combined an OCT system operating at 1300nm that enables high-speed and high-resolution transscleral imaging of the CM to an existing custom made SD-OCT platform at 840nm for dynamic biometry of the human eye. An algorithm was developed to correct for optical distortion of the OCT images. The OCT system produces high-resolution and high-contrast images of the CM and together with the algorithm for correcting image distortions enables correct estimation of the CM thickness during accommodation.

8930-30, Session 7

Improved in vivo imaging of human blood circulation in the chorioretinal complex with new phase stabilized 1 µm swept-source phase-variance optical coherence tomography (SSpvOCT)

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We tested the feasibility of our newly developed phase stabilized high-speed (100 kHz A-scans/s) 1 µm swept-source optical coherence tomography (SS-OCT) system with the phase-variance based motion contrast method for visualization of human chorioretinal complex microcirculation. Compared to our previously reported spectral domain (spectrometer based) phase-variance (pv) OCT system it has advantages of higher sensitivity, reduced fringe washout for high blood flow speeds and deeper penetration in choroid. High phase stability SSpvOCT imaging was achieved by using a modest and computationally efficient phase stabilization approach. This process does not require additional calibration hardware and complex numerical procedure. Our phase stabilization method is simple and can be employed in a variety of SS-OCT systems. Examples of vasculature in the chorioretinal complex imaged by SSpvOCT will be presented and compared to retinal images of the same volunteers acquired with indocyanine green angiography (ICGA). Choriodal imaging of patients with exudative age-related macular degeneration (AMD) will also be presented.

8930-31, Session 7

Motion-artifact-free multicontrast optical coherence tomography with simultaneous polarization and Doppler imaging

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We demonstrate motion-artifact-free multi-contrast optical coherence tomography (MC-OCT) with 200 kHz A-line rate and sample motion triggered adaptive scanning, and it enables simultaneous polarization sensitive and Doppler imaging.

In order to increase A-line rate, an optical buffering was implemented with fiber spool and fast fiber optical switch. The buffered and non-buffered spectral interference signals are suffered by different amount of system dispersion and have different shape of its envelope. And hence the buffered and non-buffered spectral sets were independently processed for MC-OCT images formation including phase stabilization, dispersion correction and system birefringence correction. Our MC-OCT enables simultaneous polarization sensitive 4-channel detection and they correspond to Jones matrix elements. In our scanning protocol, 4 B-scans are performed at a single location. And a MC-OCT image set, scattering OCT, Doppler phase, cumulative phase retardation, and degree of polarization uniformity images, are calculated from 4 Jones matrixes.

Even with high-speed A-line rate, still involuntary quick eye motion could be occurred during a volume measurement. In our retinal scanner, a

CMOS camera is equipped for pupil monitoring to aid subject alignment and spatial summation of the intensity difference between successive pupil images is monitored for eye motion detection. Once the summation of the intensity difference exceeds a pre-defined threshold, scanning probe beam is pulled-back to several B-scan locations to recover the artifact. A motion artifact suffered Doppler en face projection image is stripped according to artifact lines and registered into single image. Finally a motion-artifact-free image is obtained with a minimum scanning time.

8930-32, Session 7

In vivo imaging of the choriocapillaris using ultrahigh-speed swept source OCT angiography

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Choroidal blood flow, the largest source of blood supply to the posterior eye, is responsible for supplying oxygen to the outer retinal layers as well as metabolic transport from the retinal pigment epithelium (RPE). Several histological and electron micrograph corrosion casting studies have found that the choriocapillaris is associated with disease progression, in age-related macular degeneration and diabetic retinopathy, major causes of vision loss and impairment. To date, most studies of the choriocapillaris have been limited to post mortem studies and therefore the ability to visualize choriocapillaris in vivo is of great interest. In this study, we investigated imaging the choriocapillaris microvasculature using ultrahigh speed swept source OCT speckle decorrelation angiography with a 400kHz A-scan rate VCSEL swept light source at 1060nm wavelengths. OCT angiograms of the choroidal vasculature were generated by detecting motion contrast between OCT intensity images repeatedly acquired rapidly from the same location on the retina. Volumetric OCT angiograms were segmented at the RPE to visualize the choroidal vasculature in an en face plane. Three-dimensional angiograms enabled visualization of the choriocapillaris, feeding arterioles and draining venules in the Sattler's layer, and the interconnection between the capillaries and larger vessels. OCT angiograms at different fundus locations and depths were consistent with histological studies. As a non-invasive in vivo imaging technique, this method will enable longitudinal as well as cross-sectional studies in patients, and may be useful for understanding pathogenesis, early diagnosis of retinal diseases, monitoring treatment response, and pharmaceutical development.

8930-33, Session 7

Visualization of transretinal blood flow in retinal angiomatous proliferation with phase-resolved optical frequency domain imaging

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Retinal angiomatous proliferation (RAP) is a distinct form of neovascular age-related macular degeneration (AMD) characterized by intraretinal neovascularization (IRN) and progression to retinal–choroidal anastomosis (RCA). RAP responds better to combination therapy with PDT than to anti-VEGF monotherapy which is the standard treatment for the more common classic or occult choroidal neovascularizations in AMD. To optimize treatment strategies it is important to easily and accurately distinguish RAP lesions from other forms of neovascular AMD. Current diagnostic tools, such as fluorescein and indocyanine-green angiography (FA, resp. ICG) and conventional optical coherence tomography (OCT) are not always able to accurately identify an RAP. Angiography with OCT is a non-invasive and potentially ideal tool to evaluate retinal and choroidal vasculature, particularly in RAP. OCT-angiography is commonly referred to as Doppler or phase-resolved OCT in which (blood)flow can be visualised by detecting the phase-difference between two successive A-scans. Three treatment-naïve RAP patients were imaged with an experimental phase-resolved optical frequency domain imaging system (PR-OFDI). We present, for the first time, PR-OFDI measurements representing blood flow of IRN and RCA in RAP. In all patients the depth location of the IRN's was clearly visualized. A high similarity was found between the vasculature displayed on PR-OFDI images and on FA. PR-OFDI may be complementary to the conventional diagnostic tools because it enables easy, safe and repetitive examination of patients. This preliminary data in RAP patients using angiography with phase-resolved OCT shows a significant clinical potential of non-invasively visualizing and localizing transretinal blood flow in these patients.

8930-34, Session 8

Quality control of human graft corneas with full-field optical coherence tomography

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Eye banks currently lack an efficient method for the study of epithelium and stroma of human graft corneas. Corneas are qualified for graft on the basis of endothelial examination by conventional optical microscopy and patient history. We evaluated the performance of a full-field optical coherence tomography (FF-OCT) system for improved quality control of graft corneas, and compared visibility of features in FF-OCT images with standard spectral-domain OCT prior to keratoplasty and gold-standard histology.

Full-field optical coherence tomography offered a non invasive method of obtaining three dimensional images at ultrahigh resolution (1um in all directions) comparable to traditional histological sections. Images were acquired on human donor corneas (in normal and oedematous conditions) and surgical specimens of pathological corneas (Fuchs dystrophy, keratoconus, stromal keratitis).

FF-OCT images provided a precise visualisation of the cells and the different structures (epithelium, Bowman's membrane, stroma, Descemet's membrane and endothelium) in normal corneas, but also in pathological corneas (even in the presence of an oedema), with specific

lesions in each condition.

FF-OCT, thanks to its complete non invasive anatomical study of the cornea, could be helpful in the evaluation and selection of human cornea grafts whose quality plays a major role in the outlook of the corneal transplant.

8930-35, Session 8

Manual dynamic tracking for microscope-integrated OCT in ophthalmic surgery

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Intrasurgical optical coherence tomography (OCT) provides ophthalmic surgeons with direct depth information, eliminating uncertainty from indirect depth cues. Microscope integration of OCT (MIOCT) further allows simultaneous use of OCT with the surgical microscope. Though the two systems are coaxial and share a focal plane, the microscope field of view is typically larger than the OCT field of view. To ensure that OCT scanning can be rapidly moved to regions of interest in the larger microscope field of view, we implemented manual dynamic tracking. The manual dynamic tracking consisted of a dedicated computer receiving a live video feed of the surgical field of view. Movement of the mouse on this system delivered corresponding voltage offsets to the galvanometer mirrors resulting in centering the OCT scan on the location of the mouse cursor. Manual dynamic tracking MIOCT was successfully used in 5 ocular anterior segment surgeries to quickly reposition the OCT scanner to regions of interest within the larger microscope field of view. In contrast to repositioning the surgical microscope or acquiring a large volumetric scan in hopes of imaging the region of interest, manual dynamic tracking MICOT provides valuable surgical information from OCT without disrupting the surgery. Future implementations will use image processing to track surgical instruments in lieu of manual mouse input to move the location of the OCT scan within the surgical field.

8930-36, Session 8

Intraoperative OCT for lamellar ocular surgery

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Optical coherence tomography (OCT) has become an indispensable diagnostic device in ophthalmology. The unique possibility to provide cross-sectional or 3D images of ocular structures with 10 µm depth resolution does not only improve diagnosis but can also improve ocular surgery. With a newly developed OCT camera, which attaches to the camera port of a Haag-Streit surgical microscope, the anterior and posterior section of the eye could be visualized during ophthalmic surgery. Being completely integrated in the workflow, intraoperative OCT (iOCT) enables the surgeon to visualize virtually all tissue structures, which are accessible to diagnostic OCT. Attached to a Hi-R Neo 900A NIR, the OCT camera provides images with a lateral resolution between 10.3 ?m and 20.8 ?m (depending on selected magnification) and 7.5 ?m axial resolution (inside the tissue) at 10 Hz frame rate.

Descemet membrane endothelial keratoplasty (DMEK) is an innovative but demanding surgical technique, which selectively replaces diseased endothelium. With iOCT each step of the surgical DMEK procedure can be controlled. Stripping of the grafts and rolling behavior was monitored. The endothelial side was exactly identified. Graft localization within the anterior chamber after air filling and the final attachment to the cornea were successfully controlled.

Although more than 15 years ago the first clinical OCT device was introduced, only recently OCT was made available to the ophthalmic surgeon. Numerous application of iOCT can be envisioned in today's ophthalmic surgery. In DMEK iOCT has shown to be a valuable support in all steps of the procedure. Especially less experienced surgeons may benefit. It is expected, that iOCT will lead to improved outcome and reduced procedure duration.

8930-37, Session 8

Repetitive magnetic stimulation improves retinal function in a rat model of retinal dystrophy

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Purpose: To evaluate the effect of repetitive magnetic stimulation (RMS) on retinal functions in Royal College of Surgeons (RCS) rats.

Methods: Four weeks-old RCS and control Spargue Dawley (SD) rats underwent RMS treatment (12 sessions for 4 weeks) at intensity of either 150% or 100% of the mean resting motor threshold (MRMT, 8 RCS and 5 SD rats or 13 RCS and 3 SD rats, respectively) over the right eye. 14 RCS and 8 SD rats received sham treatment as control. Retinal functions were examined weekly by electroretinogram (ERG) under dark and light adaptation prior to treatment and for 7 weeks following end of treatment. H&E histopathology analysis was used for assessing retinal structure.

Results: RMS treatment at intensity of 150% of the MRMT significantly increased ERG b-wave responses by up to 6-fold or 10-fold in the left and right eye respectively, 3-5 weeks following end of treatment. This treatment resulted in a short and transient reduction in ERG response in the right eye, 2 weeks following end of treatment. RMS treatment at intensity of 100% of the MRMT significantly increased ERG negative wave response by 5 fold 5 weeks following end of treatment with no adverse effect on ERG response or retinal structure of SD rats.

Conclusions: RMS treatment induces delayed improvement of retinal functions in a rat model of retinal degeneration. These results suggest that RMS treatment may induce neural plasticity in the retina. This non-invasive treatment may possibly be used in the future as a primary or adjuvant treatment for retinal dystrophy.

8930-38, Session 8

Transscleral selective laser trabeculoplasty (SLT) without a gonioscopy lens

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Purpose: To evaluate whether direct transscleral application of SLT irradiation to the perilimbal area is effective in reducing Intraocular Pressure (IOP), eliminating the need for gonioscopy during the procedure.

Methods: A randomized, masked, controlled trial was performed on open angle and pseudoexfoliative glaucoma patients. The control group underwent conventional SLT delivering 100 laser spots through a gonioscope for 360 degrees directly on the trabecular meshwork (TM). The trial group underwent irradiation by the same laser at the same irradiation parameters. A similar number of applications were administered all around the limbus on the sclera overlying the TM. IOP and adverse events were measured for 6 months.

Results: In the trial group (N=11), IOP decrease from an average of 20.90 mmHg before treatment to 15.89 at 2 months and 15.00 at 6 months. The corresponding numbers for the control group (n=10), were 20.50mmHg, 14.71 and 7 (one patient) respectively. There was no statistical difference between the two groups in IOP reduction. Success, defined as >20% IOP

reduction, was attained in 7 patients of each group without a statistically significant difference between the groups [P= 0.757, Fisher].

Conclusions:. Laser coherency, lost in tissue transmission, is not required for the therapeutic effect and the mechanism of action of the external laser irradiation studied is probably similar to that of the conventional one. It seems that gonioscopy is not necessary for SLT. The novel method will simplify and shorten the SLT procedure considerably, eliminate the corneal and gonioscopy-induced side effects and may enable treatment of angle closure glaucoma.

8930-39, Session 8

Non-damaging laser therapy of the macula: titration algorithm and tissue response

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Photocoagulation of the macula typically results in permanent scarring and scotomata, which preclude treatments in the fovea and limit the re-treatments. Non-damaging approaches to laser therapy have been tested in the past, but the lack of reliable titration and slow treatment paradigms limited their clinical use. We developed and tested a titration algorithm for sub-visible and non-damaging treatments of the retina with pulses sufficiently short to be used with pattern laser scanning. The algorithm based on Arrhenius model of tissue damage optimizes the power and duration for every energy level, relative to the threshold of lesion visibility established during titration (and defined as 100%). Experiments with pigmented rabbits established that lesions in the 50-75% energy range were invisible ophthalmoscopically, but detectable with Fluorescein Angiography and OCT, while at 30% energy there was only very minor damage to the RPE, which recovered within a few days. Patients with Diabetic Macular Edema (DME) and Central Serous Chorioretinopathy (CSC) have been treated over the edematous areas at 30% energy, using 200 um spots with 0.25 diameter spacing. No signs of laser damage have been detected with any imaging modality. In CSC patients, subretinal fluid resolved within 45 days. In DME patients the edema decreased by approximately 150um over 60 days. After 3-4 months some patients presented with recurrence of edema, and they responded well to retreatment with the same parameters, without any clinically visible damage. This pilot data indicates a possibility of effective and repeatable macular laser therapy below the tissue damage threshold.

8930-40, Session 9

GPU accelerated realtime wavefront sensorless AO-OCT for in vivo small animal imaging

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Adaptive optics (AO) have been integrated with Scanning Laser Ophthalmoscopy (SLO) and Optical Coherence Tomography (OCT) for aberration correction when performing retinal imaging through a large pupil. Integration of AO with SLO and OCT for small animal imaging is burdensome due to the reflection of the wavefront sensing beacon from multiple strongly scattering layers in the mouse retina. We present a sensorless AO-OCT system that eliminates these issues in wavefront sensing by using the image quality data directly for AO correction. Furthermore, our sensorless AO approach facilitates the use of a lens-based OCT system, which greatly reduces the system complexity. A GPU processing platform was used to accelerate OCT processing for real time extraction of intensity information from specific retinal layers in the acquired volume. A modal approach of optimizing Zernike terms on the segmented MEMS based deformable mirror (IrisAO) was used in combination with an adaptive search algorithm for rapid convergence. Images of mouse retina acquired using our AO-OCT system in vivo, demonstrating the improvement in image brightness and feature sharpness when using sensorless AO. Furthermore, we demonstrated arbitrary selection of retinal layers for AO correction which is not possible with standard AO system relying on wavefront measurements.

8930-41, Session 9

Method to noninvasively probe the modal content of cone photoreceptors

Zhuolin Liu, Omer P. Kocaoglu, Timothy L. Turner, Donald T. Miller, Indiana Univ. (United States)

Vision starts with the capture and absorption of photons by photoreceptors, a process that has attracted increased clinical interest as it can reveal the stage and degree of various retinal abnormalities. Motivated by these clinical findings, extensive theory has been developed to model light capture in photoreceptors based on the principles of optical waveguiding. While powerful, the models remain largely abstractions, owing to the lack of experimental tests of their predictions of optical modes. To test for modal behavior of cones, we propose a noninvasive method that takes advantage of the high 3D resolution of adaptive optics optical coherence tomography (AO-OCT) and uses tight focus control to excite modes, fine spatial sampling to preserve mode detail, and high B-scan rates to minimize eye motion artifacts. To evaluate, the AO-OCT beam was systematically focused over a narrow range (0.15 Diopters) in steps of 0.025 Diopters in the photoreceptor layer. At each step, volumes of a 0.5 deg?0.5 deg retinal patch at 6 deg temporal retinal eccentricity were acquired using 0.6 microns/pix A-scan sampling and 644 Hz B-scan rate. Waveguided reflections were extracted at layers corresponding to the inner-segment outer-segment junction and posterior tip of outer segment. Analysis of these reflections indicate that cones at the examined location have outer segments that support only one mode and inner segments that support multiple modes. AO-OCT with tight focus control and dense sampling provides a novel approach to noninvasively probe the modal content of photoreceptors.

8930-42, Session 9

Adaptive optics optical coherence tomography for automated temporal analysis of cone photoreceptors

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Adaptive optics optical coherence tomography (AO-OCT) is an attractive noninvasive method for three dimensional imaging of cone photoreceptors and for investigating optical biomarkers that correlate with their physiology. Such time-based markers, however, are difficult to track over a large number of cones and over time due to eye motion. AO-OCT and a novel algorithm were combined to image cones over an entire day, to automatically identify and register cones, and to quantify temporal changes of three biomarkers: reflectance of the two brightest cone layers and length of the cone outer segments. The 2nd-generation Indiana AO-OCT system, with image acquisition parameters optimized for cone imaging (3vol/s at 200,000Alines/s), acquired volumes of 0.7degx0.7deg retinal patches at 3deg temporal to the fovea of a subject every hour for 15 hours with dynamic AO correction. A custom algorithm segmented and identified inner-segment outer-segment junctions (IS/OS) and posterior tips of outer segments (PTOS); registered volumes to one another using projected en face images; and tracked cones over the 15 hours. For each cone and time point, OS length and normalized reflectance of IS/OS and PTOS were measured. 733 cones

were successfully tracked across volumes and analyzed. Average OS length fluctuated by just 0.7microns peak-to-valley (with 0.1micron precision) over the 15 hours. On average, PTOS was 40% brighter than IS/OS, and slightly more unstable (4.7% compared to 3.9% RMS, with <1% precision). Small, but significant, fluctuations in OS length and reflectances were found, indicating the necessary precision to investigate optical correlates of cone physiology was achieved.

8930-43, Session 9

Photoreceptor phantom for evaluation of adaptive optics scanning laser ophthalmoscopy

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The future of ophthalmic imaging devices will include adaptive optics (AO) in one form or another. One critical issue that has limited widespread adoption of AO for routine clinical use is the variability of research-grade and clinical-prototype devices, especially with respect to image quality. One potentially beneficial tool to accomplish better assessment of system performance is a custom AO eye phantom. An optimally designed phantom should have two components: one that models the dynamic and personalized aberrations of an eye that mostly arise from the anterior ocular elements (i.e., the focusing elements – tear film, cornea, and lens), and another that models the heterogeneous appearance of the retina layers. We will present some designs for the former component but the majority of initial work discussed will be focused on the latter component. In particular, we sought to begin development of a photoreceptor phantom that mimics certain retinal anatomy. We report preliminary work to fabricate a model photoreceptor mosaic, which could be used to objectively evaluate the accuracy of cone counting software. We demonstrate the capability to vary the size of the photoreceptors via manipulation of laser parameters, most notably the pulse energy. This study will help provide the FDA with the tools to objectively evaluate the safety and efficacy of this emerging ophthalmic technology.

8930-44, Session 9

Compact adaptive optics line scanning retinal imager: closer to the clinic

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PSI's recently developed compact Adaptive Optics Line-Scanning Ophthalmoscope (AO-LSO) system is designed for rapid, automated generation of cone photoreceptor density maps. The system combines the power of AO correction with PSI's patented LSO technology. The device has a compact foot-print suitable for clinical deployment. This allows clinicians and researchers to resolve and rapidly map photoreceptors for screening or exploring retinal structures with high resolution. The system previously presented at Photonics West has been upgraded to include numerous new features that support clinical research applications, including: a pupil camera for quick patient alignment (x, y and z); a point-spread function monitoring camera for automated calibration and a convenient AO-correction and image quality metric; AO focus control for layer selection and programmed focus; dual-scanner controls with multiple modes for automated montage acquisition; WS trigger delay and integration control for best AO correction in desired ROIs; isoplanatic patch control for maximizing diffraction-limited imaged area; auto-align and auto-calibration routines enable routine maintenance and remote support; AO-OCT mode aids selection of image plane depth and image interpretation. The imager has a new patient interface which eliminates physical motion of the imager and minimizes the need for the operator to chase subjects' head movements. No optical fibers exit the imager enclosure, eliminating possible vibration-induced power or polarization fluctuations in the illumination beams. These upgrades significantly enhance the capabilities of the AO-LSO-OCT imager, providing the clinician with simultaneously-acquired (registered) en face photoreceptor images and OCT retinal cross-sections. We present a detailed discussion of these new features.

8930-45, Session PSun

Cost-effective instrumentation for quantitative depth measurement of optic nerve head using stereo fundus image pair and image cross correlation techniques

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One of the main problems with glaucoma throughout the world is that there are typically no symptoms in the early stages. Many people who have the disease do not know they have it and by the time they find out, the disease is usually in a more advanced stage. Most retinal cameras available in the market today use sophisticated optics and have several other features/capabilities (wide-angle, red-free, angiography, etc) that make them expensive for the general practice or for screening purposes. Therefore, it is important to develop instrumentation that is fast, effective and economic, in order to reach the mass public in the general eye-care centers. In this work, we have constructed the hardware and software of a cost-effective and non-mydriatic prototype device that allows fast capturing and plotting of high-resolution quantitative 3D images and videos of the optical disc head and neighboring region (300 field of view). The main application of this device is for glaucoma screening, although it may also be useful for the diagnosis of other pathologies related to the optic nerve.

8930-46, Session PSun

Phase retardation differences in diabetic versus normal eyes

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Polarimetry is used for visualizing and quantifying changes that occur in birefringent tissues of the eye, including but not limited to the cornea and Henle fiber layer. The axons of the cone photoreceptors are displaced from the foveal center in the central macula, and exhibit systematic form birefringence in a characteristic called the macular bow-tie. Changes in these structures can be indicative of disease. Using a confocal polarimeter, we took foveal centered scans with 20 different polarization states for 12 diabetic subjects and 12 age-, gender, and race/ethnic-matched controls. Using Matlab, we calculated the phase retardation differences between these two groups over 7.5 degree field of view. Phase data were weighted pixel by pixel by the amplitude at that location, for the cross detector and parallel detector separately. The coefficient of variation of each diametric measurement, along cone axon paths, was computed. The phase varied along the diameters significantly more for the diabetics subjects as compared with the normal subjects for the cross detector ($p<0.0000003$), but not for the parallel detector ($p=0.968$). Half of the diabetics have a mean weighted coefficient of variation that is significantly greater than the cut-off as determined by the normal subjects used in this study.

8930-47, Session PSun

UV protection for sunglasses: revisiting the standards

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In a continuing work of establishing safe limits for UV protection on sunglasses, we have theoretically estimated the incident UV radiation for the 280nm–400nm range for 5500 locations in Brazil. Current literature establishes safe limits regarding ultraviolet radiation exposure in the spectral region 180nm–400nm for weighted and unweighted UV radiant exposure. British Standard BSEN1836(2005) and American Standard ANZI Z80.3(2009) require the UV protection in the spectral range 280nm–380nm, and The Brazilian Standard for sunglasses protection, NBR15111(20013), currently requires protection for the 280nm – 400nm range as established by literature. However, none of them take into account the total (unweighted) UVA radiant exposure. Calculations of these limits have been made for 5500 Brazilian locations, which included the geographic position of the city; altitude, inclination angle of the Earth; typical atmospheric data (ozone column; water vapor and others) as well as scattering from concrete, grass, sand, water, etc. Furthermore, regarding UV safety for the ocular media, the resistance to irradiance test required on this standard of irradiating the lenses for 25 continuous hours with a 450W sunlight simulator leads to a correspondence of 26 hours and 10 minutes of continuous exposure to the Sun. Moreover, since the sun irradiance in Brazil is quite large, integrations made for the 280-400nm range shows an average of 45% of greater ultraviolet radiant exposure than for the 280-380nm range. Suggestions on the parameters of these tests are made in order to establish safe limits according to the UV irradiance in Brazil.

8930-48, Session PSun

UV transmittance during the crosslinking procedure: tunable treatment

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The transmittance of UVA light through the in vitro human cornea over the thickness of 400um during the corneal collagen cross-linking procedure has been measured using an optical fiber (600 µm core diameter) fixed just before the cornea and attached to Spectrophotometer. The 10 corneas, (average of 6 days post-mortem) were washed with saline and cross-linked with the currently used protocol. To enhance absorption of UV radiation, Riboflavin solution (0.1% and 400 mOsm) was applied prior to and during exposure. The UVA beam - 365nm ± 5nm at 3mW/cm2 ± 0.003mW/cm2 - was focused directly onto the corneal stroma. The measured average transmittance of the cornea without Riboflavin was 64.1%. Preceding the irradiation but after 6 applications of Riboflavin at 5min intervals (total of 30min) transmittance decreased to 21.1%. The 30min of irradiation were then accompanied by an additional 6 applications of Riboflavin at 5min intervals (for a total of treatment time of 1h), resulting in a further decrease in transmittance to 12.2%, which is in agreement with current literature. The average transmittance in terms of energy during the 30 minutes irradiation procedure fluctuated from 0.63 to 0.37 mW/cm2. These results indicate different levels of UV transmittance during treatment, leading to consider a new personalized treatment with tunable UV power irradiation.

8930-49, Session PSun

Flammability test for sunglasses: developing a system

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The ultraviolet radiation from the sun cause several eye diseases. Recent investigations show the need for certificating sunglasses to ensure the safety and health to population. The Brazilian Standard ABNT NBR 15111 regulates features to sunglasses, however, Brazil does not have any sunglasses certifying institution. We propose a flammability test system for sunglasses in compliance with the NBR 15111. The standard provides requirements for the flammability test procedure: the equipment must operate at a temperature of 650 °C ± 20 °C; the end of a steel rod of 300 mm length and 6 mm diameter should be heated and pressed over the surface of the lenses for five seconds; the flammability is checked by visual inspection. The furnace is made of ceramic. We used a power electronic circuit to control the power in the furnace using on-off mode and for measuring the temperature, we used a K-type thermocouple. A stepper motor with pulley lifts the steel rod. The system reached the working temperature in 19 minutes for a step input of 61 V in open loop system. The electronics control are under development in order to shorten the time necessary to reach the working temperature and maintain the temperature variation in the furnace within the limits imposed by the standard as next steps. The proposed system is pioneer in Brazil. Moreover, the equipment is part of the first Brazilian certification laboratory for sunglasses, the CERTIFICA-LIO. To guarantee safety and health to sunglasses customers, this research represents opportunities for futures discoveries.

8930-50, Session PSun

Detection of chronologic changes in the size of human choroidal vascularization using 1-micron swept-source optical coherence tomography and a new semi-automated system

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Various macular diseases are thought to originate from choroidal vascular abnormalities. We found that the choroidal thickness physiologically changes in the short term based on longitudinal optical coherence tomography (OCT) observation. We measured the choroidal vascular area in sequential OCT images.

Two healthy volunteers were enrolled. The center wavelength of swept-source OCT was 1,050 nm, and the image acquisition rate was 100 K. B-scans with 1,024 A-scans were obtained sequentially at 86 frames per second. One observer selected a vessel and its central position from the first B-scan. The extracted image was converted from Cartesian to polar coordinates and then segmented. The image was re-converted, and the area surrounded by these points was calculated. The frequency was assessed using fast Fourier transform (FFT).

The entire vessel area changed over time and the range of fluctuations varied greatly. Some wave components had a frequency similar to that of the pulse. FFT showed the vessels had either a small or large amplitude pattern. The integral of the FFT graph area confirmed the two patterns.

The choroidal vessels reportedly have pulsatile reactions, and our results were consistent, although we could not rule out optical/mechanical bias. We found two patterns of vascular reactions with different amplitude profiles that may have resulted from the different characters of the vessels. The new semi-automated measurement of the choroidal vessel size using polar coordinates seems to work. This system will facilitate more extensive choroidal vascular analysis in the future.

8930-51, Session PSun

Robotic console for ocular surgery: a preliminary study

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Minimally invasive surgery has recently been improved by the use of robot-assisted procedures in several medical fields. Among the ocular surgeries there are a few examples of sophisticated vitreoretinal procedures, while robotic-assisted surgery of the anterior eye segment is still under study. In this paper we propose a new approach to the robotic assisted ocular surgery. An optimized Cone Beam CT is used to study and to visualize the ocular area, by the use of a 3D image reconstruction. A specialized software is used to transfer spatial information to a robotic arm, that can be used to move different end-effectors. A sensorized tool is connected to the patient eye and to the robotic arm. This tool is equipped with force and position sensors: by the use of the spatial information from the robotic console and from the patient it is possible to control the position of the target itself and to block it in the correct position for performing surgery. The system is provided of a feedback alarm that remove the block of the patient head in any moment. The optimized robotic console can be used in the preoperative design of the surgery, for the real time control in the surgery room and to move specialized end effectors for ocular surgery, such as a fiber optic delivering a laser light (e.g. in the laser welding of the tissues), the positioning of retinal or corneal prosthesis, the trauma surgery, the onco-ophthalmological applications (precise removing of tumor tissue, positioning of ruthenium plaques).

8930-52, Session PSun

Femtosecond laser assisted design of sutureless intrastromal graft as an alternative to partial thickness keratoplasty

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Minimally invasive laser assisted surgery is continuously developing in order to find new surgical approaches, preserving the patient tissue and improving surgical results in terms of cut precision, restoration of visual acuity, and invasiveness. In order to achieve these goals, the current approach in corneal transplant is lamellar keratoplasty, where only the anterior or posterior part of the patient's cornea is substituted, depending on the lesion or pathology. In this work we present a novel alternative approach: a case study of intrastromal sutureless transplant, where a portion of the anterior stroma of a donor cornea was inserted in the stroma of the recipient cornea, aiming at restoring the correct thickness of the patient's cornea. The patient cornea was paracentrally thin, as the result of a trophic ulcer due to rheumatoid arthritis. A discoid corneal graft from the anterior stroma of a donor eye was prepared: a femtosecond laser cut with a trapezoidal profile (thickness was 300 µm, minor and major basis were 3.00 and 3.60 mm, respectively). In the recipient eye an intrastromal cut was also performed with the femtosecond laser, by the use of a specifically designed mask; the cut position was 275 µm in depth. The graft was inserted onto an injector and inserted as an intrastromal presbyopic implant. The postoperative analysis evidenced a clear and stable graft that selectively restored corneal thickness in the thinned area. Intrastromal corneal transplant surgery may be considered as a possible minimally invasive alternative to anterior or posterior lamellar keratoplasty in selected cases.

8930-53, Session PSun

Utilization of the excimer laser and a moving piezoelectric mirror to accomplish the customized contact lens ablation to correct high-order aberrations

Luciana de Matos, Wavetek Technologies Insustry Ltd. (Brazil) and Univ. de São Paulo (Brazil); Fatima M. Yasuoka, Univ. Federal de São Paulo (Brazil) and Wavetek Technologies Industry Ltd. (Brazil); Paulo Schor, Enos Oliveira, Univ. Federal de São Paulo (Brazil); Vanderlei S. Bagnato, Univ. de São Paulo (Brazil); Luis Albert V. Carvalho, Wavetek Technologies Industry Ltd. (Brazil) and Univ. de São Paulo (Brazil)

The use of Hartman-Schack sensor in Ophthalmology allowed the identification of higher-order aberrations, which make possible the research for methods to correct them. Customized refractive surgery is one of the most successful methods, although there are patients cannot be submitted to this surgery due to a variety of abnormal limiting factors such as cornea thickness and quantity of higher-order aberrations. Being this an irreversible process, the alternative is to develop a non-surgical method. This work proposes the development of a method using excimer laser and moving piezoelectric mirror to perform the customized contact lens ablation to correct high-order aberrations. The process to produce such lens consists of four steps. 1) The map of total aberrations of the patient's eye is measured by using an aberrometer with a sensor de Hartman-Shack. 2) The measure aberration map is used to determine the related correction map, which is used to define the pulse distribution map, to be produced via ablation with excimer laser. 3) The lens production must be performed as the same principle of customized refractive surgery. 4) The quality control of the lens is evaluated by two tests. 4.1) The lens is measured by a non-commercial lensometer, which is assembled specially for this measurement, due to ones commercially available are not capable to measure asymmetric and irregular surface. 4.2) The evaluation of the lens-eye system is made using the aberrometer of the first step in order to verify the residual aberrations. These lenses are ablated with a customized refractive surgery system.

8930-54, Session PSun

Development of a universal toric intraocular lens calculator

David Hjelmstad, Arizona State Univ. (United States) and The Eye Center (United States); Samir I. Sayegh M.D., The Eye Center (United States)

We present a method for calculating the ideal toric lens to implant in astigmatic patients following cataract surgery, and an implementation of this calculator. We show that the online calculators provided by the two main toric IOL manufacturers, Alcon and Tecnis, are insufficient for both theoretical and practical reasons. We have reverse-engineered the algorithm used by Alcon for this calculation, and in doing so have revealed important theoretical shortcomings in the approximations they utilize in the calculation. The clinical severity of these shortcomings is illustrated by a number of cases which illustrate how the approximations made can lead to unnecessary errors in lens selection. Our approach combines the spherical and cylindrical power calculations into one, eliminating the reliance on separate programs to conduct this computation. Furthermore, the implementation allows for lens data to be entered regardless of the manufacturer, allowing for the determination of the optimal lens among all available lenses, and eliminating the need to use separate calculators for separate manufacturers.

8930-55, Session PSun

Repetitive magnetic stimulation reduces corneal permeability

Ygal Rotenstreich, Tel Aviv Univ. (Israel); Avner Belkin, Meir Medical Ctr. (Israel); Sapir Kalish, Ifat Sher, Michael Belkin, Tel Aviv Univ. (Israel)

PURPOSE: magnetic stimulation (RMS) on corneal permeability in a short-term dry eye model.

METHODS: Rabbits underwent RMS treatment on the right eye at intensities of 50, 100 and 130% of the mean resting motor threshold (MRMT). One hour post treatment, rabbits were anesthetized and eyes were held open with an eye specula for 3 hours. Five microliters of 0.5% fluorescein solution was applied to the cornea in untouched fashion. Thirty seconds after instillation, the ocular surface was extensively washed. Sixty minutes afterward, corneas were photographed with a slit lamp biomicrophotography with subsequent computerized area determination of the size of the epithelial defect. In addition fluorescence intensity in aqueous humor obtained by anterior chamber tap taps (100 microliters) was measured and converted into fluorescein concentration.

RESULTS: In rabbits treated with RMS at 100 and 130% MRMT, the size of epithelial defect was significantly reduced when compared with the sham-treated eyes. There was no significant difference in the size of epithelial defect between eyes treated with RMS at 66% MRMT and sham-treated eyes. Eyes treated with RMS at 100 and 130% MRMT demonstrated a 2- and 3-fold reduction, respectively in fluorescein concentration in the aqueous humor when compared with the sham-treated eyes. By contrast, there was no significant difference in fluorescein concentration in the anterior chamber tap between eyes treated with RMS at 66% MRMT and sham-treated eyes.

CONCLUSIONS: Our findings suggest that RMS treatment can minimize barrier defects arising from epithelial damage, suggesting that RMS may be used for treatment in patients with compromised corneal epithelium.

Conference 8931: Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXIII

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8931 Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy XXIII

8931-1, Session 1

Increased efficacy of photodynamic therapy via sequential targeting *(Invited Paper)*

David H. Kessel, Neha Aggarwal, Wayne State Univ. (United States); Bonnie F Sloane, Wayne State Univ (United States)

Mitochondria are among the more potent targets for PDT, since release of cytochrome c can directly activate the apoptotic death pathway. We have examined phenomena associated with mitochondria photodamage with a view toward optimizing protocols. Loss of the mitochondrial membrane potential (??m) accompanies mitochondrial photodamage but this was not necessarily correlated with photokilling and was found to be reversible, even in lethally-damaged cells. A low level of lysosomal photodamage could markedly promote photokilling by subsequent mitochondrial photodamage to 1c1c7 murine hepatoma cells in 2-D culture, and in human-derived inflammatory breast cancer cells in 3D culture. This effect was not associated with enhanced formation of singlet oxygen or OH radicals. The results are, however, consistent with an initial generation of a strong pro-apoptotic signal that can amplify the effects of mitochondrial photodamage.

8931-2, Session 1

Drug resistance mechanisms, photodynamic therapy (PDT) and combination treatment *(Invited Paper)*

Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

Resistance of cancer cells to treatment, be inherent or acquired, is multifactorial and may be viewed in two broad categories. The first arises from alterations within cancer cells that inhibit the action of drugs through a variety of mechanisms such as enhanced drug efflux or inhibition of cellular repair machinery. The second originates in alterations (increase or decrease) of signaling pathways that regulate cell growth and proliferation where there is also a possibility of cross talk, thus creating additional challenges in the destruction of cancer cells. Photodynamic therapy may have a specific role in ameliorating drug resistance mechanisms by the targeted destruction of such specific pathways. A combination of mechanistic understanding and creative chemistry to achieve multiagent delivery or other methods to inhibit several pathways and activate death mechanisms has the potential to enhance tumor control. Recent results on the use of such delivery constructs and approaches for PDT and their implications will be presented.

8931-3, Session 1

Delineation of the molecular mechanisms behind improved benefit following combination erlotinib/photodynamic therapy

Shannon M. Gallagher-Colombo, Rensa Chen, Joann Miller, Theresa M. Busch, Univ. of Pennsylvania (United States)

Numerous aggressive cancers are characterized by overexpression or overactivation of the epidermal growth factor receptor (EGFR), making this receptor a favored target for therapeutic intervention. Inhibition of EGFR is most commonly achieved through the use of anti-EGFR antibodies (ie – cetuximab) or small molecule inhibitors (ie – erlotinib); however, the efficacy of these molecular targeting agents is often limited by innate and acquired resistance to these drugs. Recent work from our lab has highlighted the benefit of combining photodynamic therapy (PDT) with erlotinib in tumor xenografts of human head and neck squamous cell carcinoma (SQ20B) where erlotinib combined with PDT leads to improved tumor responses. Notably, we have observed a similar therapeutic effect in the erlotinib resistant human lung cancer cell line, H460. Therefore, we sought to understand the molecular mechanisms that underlie this enhanced therapeutic benefit in erlotinib-sensitive and erlotinib-resistant tumors, with the goal of identifying either common or diverging pathways that could explain the improved responses. We explored the effect of combination therapy on activation of signaling pathways induced by EGFR, including PI3K/Akt and MAPK/Erk. We also evaluated the impact of combination therapy on expression of other transcription factors that are important for tumor progression. The results from this work will provide insight into understanding how erlotinib sensitivity can be enhanced in an effort to improve therapeutic responses in not only erlotinib sensitive, but also erlotinib insensitive malignancies.

8931-4, Session 1

Combination of photodynamic therapy and cancer molecular targeted agents

Bin Chen, Univ. of the Sciences in Philadelphia (United States)

Photodynamic therapy (PDT) induces cell damage and even cell death through the generation of reactive oxygen species (ROS). Depending on the type of cells, photosensitizer and light doses, PDT has been shown to induce cell apoptosis, necrosis and autophagy. PDT is also known to activate cell survival pathways. The final PDT outcome is dependent on the interplay between PDT-induced cell death and survival signals. To enhance the therapeutic outcome of PDT, it is necessary to further potentiate PDT-induced death signal and/or inhibit PDT-induced survival signal.

To achieve this goal, we combined PDT and molecular targeted anticancer agents in this study. Because PDT induces cell death by generating oxidative proteins which can be detoxified by proteasomes, it is hypothesized that PDT in combination with proteasome inhibitor bortezomib will increase cell death by inducing the accumulation of oxidative proteins. Based on the finding that sub-lethal PDT induced a significant upregulation of phosphorylated AKT, a pro-survival cell signal, we hypothesize that PDT in combination with phosphatidylinositol 3-kinase (PI3K) pathway inhibitor BEZ235 will increase PDT outcome by preventing cell regrowth.

We first tested these hypotheses in SVEC endothelial and PC-3 prostate cancer cell lines. PDT induced a rapid apoptosis in the SVEC4 cells, whereas a significantly delayed cell death was observed in the PC-3 cells. Combination of PDT and bortezomib or BEZ235 was found to increase cell death and growth inhibition. However, this effect was highly dependent upon cell type. SVEC4 cells were more responsive to the combination therapy than PC-3 cells in both cases. We also evaluated the effects of combination therapy in PC-3 tumor models. In agreement with the in vitro study, we found that the combination therapy significantly enhanced tumor vascular damage induced by PDT. Therefore, our present results indicate that PDT in combination with proteasome inhibitor bortezomib or PI3K inhibitor BEZ235 leads to enhanced cell death and the inhibition of cell proliferation in both in vitro and in vivo studies. It appears that enhanced damage to tumor vasculature is mainly responsible for the enhanced therapeutic outcome.

8931-5, Session 1

Metal-based phthalocyanines as a potential photosensitizing agent in photodynamic therapy for the treatment of melanoma skin cancer

Kaminee Maduray, Durban Univ. of Technology (South Africa) and Durban Univ. of Technology (South Africa); B. Odhav, Durban Univ. of Technology (South Africa)

Metal-based phthalocyanines currently are utilised as a colorant for industrial applications but their unique properties also make them prospective photosensitizers. Photosensitizers are non-toxic drugs, which are commonly used in photodynamic therapy (PDT), for the treatment of various cancers. PDT is based on the principle that, exposure to light shortly after photosensitizer administration predominately leads to the production of reactive oxygen species for the eradication of cancerous cells and tissue. This in vitro study investigated the photodynamic effect of indium (InPcCl) and iron (FePcCl) phthalocyanine chlorides on human melanoma skin cancer cells. Experimentally, 2 x 104 cells/ml were seeded in 24-well tissue culture plates and allowed to attach overnight, after which cells were treated with different concentrations (2 µg/ml – 100 µg/ml) of InPcCl and FePcCl. After 2 h, cells were irradiated with constant light doses of 2.5 J/cm2, 4.5 J/cm2 and 8.5 J/cm2 delivered from a diode laser (? = 661 nm). Post-irradiated cells were incubated for 24 h before cell viability was measured using the MTT Assay. At 24 h after PDT, irradiation with a light dose of 2.5 J/cm2 for each photosensitizing concentration of InPcCl and FePcCl produced a significant decrease in cell viability, but when the treatment light dose was further increased to 4.5 J/cm2 and 8.5 J/cm2 the cell survival was less than 50% for each of the different photosensitizing concentrations of InPcCl and FePcCl. This PDT study concludes that low concentrations on InPcCl and FePcCl activated with low level light doses can be used for the effective in vitro killing of melanoma cancer cells.

8931-6, Session 2

Identifying stromal determinants of heterogeneous treatment response in 3D tumor co-cultures *(Invited Paper)*

Imran Rizvi, Sriram R. Anbil, Emma Briars, Shazia Khan, Ruth Goldschmidt, Nermina Alagic, Massachusetts General Hospital (United States); Jonathan P. Celli, Massachusetts General Hospital (United States) and Univ. of Massachusetts Boston (United States); Lawrence B. Mensah, Iqbal Massodi, Arnav Chandra, Massachusetts General Hospital (United States); Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

Cancers develop and progress in a complex milieu of cues that influence the biological characteristics and susceptibility to treatment of the resultant lesions. These microenvironmental cues originate from many sources including the surrounding matrix, diffusible growth factors and cytokines, the architectural and organizational features of the tumor, and heterotypic communications between tumor cells and stromal partners. Among the various stromal cells that warrant investigation, tumor endothelial cells (TEC) and tumor associated fibroblasts (TAF) are emerging as important biological modulators of many cancers including ovarian (OvCa) and pancreatic cancer (PanCa). Understanding the therapeutic implications of heterogeneities in response that result from crosstalk between tumor cells and stromal partners is critical to designing more effective regimens. Photodynamic therapy (PDT), which is mechanistically distinct from conventional treatments, may play an important role in the design of comprehensive treatment plans for both OvCa and PanCa. Evaluating and optimizing combination treatments, including PDT-based regimens, will require a multi-faceted approach that includes the development of more sophisticated models. Heterocellular three-dimensional (3D) tumor arrays that restore communication with stromal partners could serve as increasingly important complements to existing systems. Current findings will be presented on the impact of TECs and TAFs on the biological characteristics, and susceptibility to PDT and traditional therapies, in OvCa and PanCa 3D co-cultures.

8931-7, Session 2

PDT for targeting drug-resistance associated with stromal interactions and epithelial-mesenchymal transition in pancreatic cancer *(Invited Paper)*

Jonathan P. Celli, Massachusetts General Hospital (United States) and Univ. of Massachusetts Boston (United States); Gwendolyn M. Cramer, Dustin P. Jones, William Hanna, Ljubica Petrovic, Univ. of Massachusetts Boston (United States); Ruth Goldschmidt, Massachusetts General Hospital (United States); Yuyu Li, Chandra S. Yelleswarapu, Univ. of Massachusetts Boston (United States); Imran Rizvi, Massachusetts General Hospital (United States); Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

Cancer of the pancreas is well known to be among the most lethal of human malignancies, and is associated with a particular abundance of rigid, fibrotic stroma that plays multiple complex roles in promoting tumor growth and drug resistance. Through interactions with tumor-associated fibroblasts, the biophysical properties and noted rigidity of the stromal microenvironment may limit drug penetration and contribute to mechanosensitive regulation of growth and therapeutic response. This cellular cross talk also contributes to increased invasion/metastasis, anti-apoptotic signaling and epithelial to mesenchymal (EMT) transition associated with drug resistance in pancreatic cancer cells. In this study we examine the ability of verteporfin-based photodynamic therapy (PDT) to overcome and/or bypass these stroma-associated mechanisms of drug resistance. Using in vitro 3D pancreatic tumor-stroma co-culture models combined with particle tracking microrheology (PTM) we monitor local changes in the mechanical microenvironment. Spatially resolved PTM measurements of extracellular rigidity are correlated with growth and therapeutic response, monitored longitudinally using digital holographic microscopy (DHM) and terminally assessed by high-content quantitative fluorescence microscopy. We also examine PDT response following EMT induced by stromal interactions, which gives rise to resistance to traditional chemotherapy agents, gemcitabine and oxaliplatin.

8931-8, Session 2

Three-dimensional cell culturing by magnetic levitation for evaluating efficacy/toxicity photodynamic therapy

Luis G. Sabino, Priscila Fernanda Campos Menezes, Vanderlei Salvador Bagnato, Univ. de São Paulo (Brazil); Glauco Souza, Nano3D Biosciences, Inc. (United States); Thomas C Killian, Rice Univ. (United States); Cristina Kurachi D.D.S., Univ. de São Paulo (Brazil)

We have used three-dimensional (3D) cell culturing by magnetic levitation to generate an in vitro model for evaluating efficacy/toxicity and optimizing near infrared photodynamic therapy (PDT). Cell culturing by magnetic levitation consists of a device (The Bio-AssemblerTM) based on magnetization of cells using magnetic nanoparticle assemblies (Nanoshuttle) and levitation of the cells. In addition to addressing

many of the unmet practical challenges in 3D cell culturing, magnetic levitation fosters tissue formation with phenotypic morphogenesis and functionality. The presence of the magnetic field levitates and spatially guides cells together, therefore promoting rapid cell-cell interaction in a manner that allows cells to self-assemble, expand, and migrate in 3D. Importantly, this process of 3D cell culturing takes place without the influence of an artificial ECM, and our results have shown that cells start to generate their endogenous ECM and assemble into physiologically relevant 3D multi-cellular structures within hours of levitation. In this presentation we will demonstrate that 3D cell culturing by magnetic levitation is a valuable tool for characterizing and optimizing the PDT process in vitro, such as evaluating photosensitizer diffusion and predicting the outcome of multisession PDT dosimetry prior to in vivo experimentation. Specifically, the 3D cultures of HepG2 (liver) and MDA-MB-231 (breast) cell lines will be used to demonstrate the in vitro dosimetry using Photogem and near infra-red light. Illumination of the samples will be accomplishes using a 630 nm LED-based device called BioTable®. Finally, we will use microscopy and an iPod-based image system to evaluate cytotoxic effects caused by PDT.

8931-9, Session 2

Parameter determination for BPD mediated vascular PDT

Michele M. Kim, Univ. of Pennsylvania School of Medicine (United States); Baochang Liu, Joann Miller, Theresa M. Busch, Univ. of Pennsylvania (United States); Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States)

The cell killing mechanism of benzoporphyrin derivative monoacid ring A (BPD) is known to be predominantly apoptotic or vascular, depending on the drug-light interval (DLI). With a 3 hour DLI, necrosis develops secondary to tumor cell damage, while with a 15 minute DLI, necrosis results from treatment-created vascular damage. The purpose of this study is to examine if the different mechanisms of cell death will affect the photochemical parameters for the macroscopic singlet oxygen model. Using the RIF model of murine fibrosarcoma, we determined the four photochemical parameters (?, ?, ?, g) and the threshold singlet oxygen dose for BPD-mediated PDT through evaluation of the extent of tumor necrosis as a function of PDT fluence rate and total fluence. Mice were treated with a linear source at fluence rates from 12-150 mW/cm and total fluences from 24-135 J/cm. BPD was administered at 1mg/kg with a 15 minute DLI, followed by light delivery at 690nm. Tumors were excised at 24 hours after PDT and necrosis was analyzed via H&E staining. The in-vivo BPD drug concentration is determined to be in the range of 0.05-0.30 ?M. The determination of these parameters specific for BPD and the 15 minute DLI provides necessary data for predicting treatment outcome in clinical BPD-mediated PDT.. Photochemical parameters will be compared between 1mg/kg DLI 3 hours and 1mg/kg DLI 15 minutes.

8931-10, Session 2

Evaluation of the Photodynamic Therapy effect using a tumor model in Chorioallantoic Membrane with Melanoma cells

Hilde H. Buzza, Layla Pires, Vanderlei Salvador Bagnato, Cristina Kurachi D.D.S., Univ. de São Paulo (Brazil)

Photodynamic Therapy (PDT) is a type of cancer treatment that is based on the interaction of light (with specific wavelength), a photosensitizing agent and molecular oxygen. The photosensitizer (PS) is activated by light and reacts with oxygen resulting in the production of singlet oxygen that is highly reactive and responsible for the cell death. The Chick Chorioallantoic Membrane (CAM) model is a transparent membrane that allows visualization and evaluation of blood vessels and structural changes, where a tumor model was developed. Two induction tumor models were investigated: tumor biopsy or cell culture. It was used a murine melanoma cell B16F10 in culture and a biopsy from a xenograft tumor in hairless mouse. Two PS were tested: Photodithazine® and Photogem®, a chlorine and porphyrin compounds, respectively. Using intravenous administration, the light-drug interval was of 30 minutes, 1 and 3 hours. Illumination was performed at 630 nm and 660 nm, and the vascular and tumor response was monitored and analyzed. The PS distribution was checked with confocal microscopy. This model can be useful to study several parameters of PDT and the effect of this therapy in the cancer treatment since it allows direct visualization of its effects.

8931-11, Session 3

Development of image-guided targeted two-photon PDT for the treatment of head and neck cancers

Charles W. Spangler, SensoPath Technologies Inc. (United States); Jean R. Starkey, Montana State Univ. (United States); Bo Liang, Sara Fedorka, SensoPath Technologies Inc. (United States); Hao Yang, Huabei Jiang, Univ. of Florida (United States)

Triad therapeutics incorporating an EGFr targeting peptide, a NIR imaging agent and a porphyrin that can be activated by two-photon excitation at 800-840 nm were used to treat FaDu HNSCC tumor xenografts in SCID mice using image guidance in the range 600-700- nm. The triad was administered by intratumoral injection and allowed to diffuse throughout the tumor for 4 hours. The targeted tumor was then imaged and irradiated at 800 nm using the tumor image to define the rastering pattern of the two-photon laser beam (900 mW, 150 fsec pulses) in 1 mm steps. An optical margin of 2 mm was added to the irradiation protocol surrounding the perceived imaged tumor volume. Robust tumor regression was observed, with 80% of the tumor destroyed in 5 days post-PDT, and complete tumor destruction 1n 15 days post-PDT treatment and rapid healing of the former tumor site. No adverse effects were observed for the PDT protocol or post-PDT.

8931-12, Session 3

Real-time monitoring of photo-immunotherapy using optical coherence tomography

Chia-Pin Liang, Yu Chen, Univ. of Maryland, College Park (United States); Takahito Nakajima, Rira Watanabe, Kazuhide Sato, Hisataka Kobayashi, Peter L. Choyke M.D., National Cancer Institute (United States)

Photo-immunotherapy (PIT) is a low-side-effect cancer therapy based on an armed antibody conjugate that induces rapid cellular necrosis after exposure to near infrared light. The conjugate consists of a hydrophilic photosensitizer phthalocyanine dye, IR700, which is covalently bound to a humanized monoclonal antibody. When exposed to near-infrared light, the conjugate induces highly selective and rapid cancer necrotic cell death, which have been demonstrated both in vitro and in vivo. Following PIT, serial histology reveals dramatic changes in the appearance of cells within 20-30 minutes. However, the gross features of the tumor are much slower to resolve. Therefore, real-time monitoring methods that detect acute changes in the tumor micro-environment will be important for understanding the mechanism of PIT treatment. In our pilot studies, optical coherence tomography (OCT) imaging reveals dramatic vascular variation during PIT. We developed and applied several techniques, including speckle variance analysis, Doppler flow measurement, bulk motion removal and automatic ROI selection to quantify the size and the flow speed of tumor vessels in vivo. The data shows the blood flow speed slow down significantly in ten minutes. This phenomenon may be caused by the dramatic change of vessel wall permeability and interstitial pressure. Lastly we demonstrate that the needle-type OCT probe can

acquire real-time imaging feedbacks, which could be used as surrogate biomarkers to modulate the NIR intensity for optimizing the therapeutic efficacy.

8931-13, Session 3

Development of photodynamic therapy for treatment of peritoneal metastases

Bryan Q. Spring, Adnan O. Abu-Yousif, Akilan Palanisami, Xiang Zheng, Imran Rizvi, Zhiming Mai, Sriram R. Anbil, R. Bryan Sears, Lawrence B. Mensah, Ruth Goldschmidt, Sultan S. Erdem, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Esther Oliva, Massachusetts General Hospital (United States); Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

Metastatic ovarian carcinoma (OvCa)—characterized by disseminated nodular studding of the peritoneal cavity—is a prime example of a frequently recurrent cancer. Recurrence stems from deposits of residual, chemoresistant cancer cells. Photodynamic therapy (PDT) has shown efficacy against chemoresistant cancer cells and promise for helping to mop up residual disease. However, clinical trials of PDT for peritoneal metastases have revealed severe dose-limiting toxicities due to non-specific photosensitizer uptake in vital tissues. This talk discusses an approach that offers promise for selective PDT of intraperitoneal disease.

8931-14, Session 3

Highly diffusive, tumor selective, molecularly targeted constructs for PDT

Oliver J. Klein, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States); Hushan Yuan, Lee Josephson, Massachusetts General Hospital (United States); Conor L. Evans, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States)

The uptake of photosensitizers by tumors is limited by delivery barriers, such as a lack of perfusion or the adherence to the extracellular matrix or fibrous capsules, leading to subcurative therapy in many instances. Approaches to improve photosensitizer accumulation in tumors, such as the use of highly cationic agents, can cause different problems such as dark toxicity (light independent toxicity). Furthermore, even when photosensitizers reach their intended destination, effects of the local environment can reduce cytotoxic potential. To overcome these problems, we synthesized photodynamic agents exploiting "PEG-photosensitizer shielding," where a photosensitizer is covalently linked to a modular peptide backbone, which in turn is coupled to a single polyethylene glycol (PEG) polymer (>2 kDa). PEG shields the photosensitizer, blocking self-association (stacking), non-specific interactions with cells or proteins, and allows unhindered diffusion through tissues. We have coupled the photosensitizer EtNBS to this modular construct, along with a cyclic cell-targeting peptide, and obtained a construct with high cellular uptake and good photodynamic activity. Importantly, when delivered to large 3D in vitro tumor models, this construct had remarkably high diffusivity and readily penetrated tumor spheroids many hundreds of microns in diameter. Current studies are focused on characterizing the cytotoxicity of this construct, in both monolayer and 3D tissue cultures, investigating photodynamic environmental screening mechanisms, and exploring its application in animal studies.

8931-15, Session 3

Monitoring of photodynamic therapy with photoacoustic imaging

Srivalleesha Mallidi, Harvard Medical School (United States); Kohei Watanabe, Massachusetts General Hospital (United States) and Canon U.S.A., Inc. (United States); Dmitriy Timerman, Tayyaba Hasan, Massachusetts General Hospital (United States)

The photosensitizer (PS) concentration at the treatment site, tumor oxygenation status and the light dose play major roles in determining photodynamic therapy (PDT) outcomes. Predominantly in PDT research, fluorescence imaging and pO2 electrodes are used to assess PS photobleaching and tissue oxygenation respectively. Fluorescence images are surface-weighted and do not provide a 3D image of the tumoral PS accumulation while measurements from the invasive pO2 electrodes do not provide information on the heterogeneous oxygenation distribution in the tumors. Moreover, these techniques do not provide simultaneous information on important PDT dosimetry parameters. Photoacoustic imaging, on the other hand, can aid in gauging the delivered PDT dose since it has the capability of imaging 3-D distribution of the photosensitizer and the blood vessel oxygenation status simultaneously. In this study we explore the utility of photoacoustic imaging in monitoring PS accumulation and photobleaching in a subcutaneous tumor murine model. The image contrast in photoacoustic imaging is based on optical absorption properties of the tissue. For example, a change in PS concentration in the tissue will lead to a change in the photoacoustic signal intensity. This information on change in photoacoustic signal at the imaging site can then be utilized to personalize PDT parameters, such as adjustment of the light dose or administration of additional PS to obtain more robust and predictable therapeutic outcome. Overall, we present the utility of photoacoustic imaging for monitoring photodynamic therapy dosimetry parameters.

8931-16, Session 3

Noninvasive tumor oxygen imaging by photoacoustic lifetime imaging integrated with photodynamic therapy

Qi Shao, Merrill A. Biel M.D., Shai Ashkenazi, Univ. of Minnesota (United States)

Oxygen plays a major role in cancer biology and tumor progression. In PDT, the reduction in efficacy is directly related to lack of oxygen because its molecular mechanism relies on oxygen as an energy mediator. Measuring tumor oxygenation can provide physicians with better diagnosis and optimization of treatment plans. However, clinical tools for directly assessing tissue oxygenation are limited. The gold standard is oxygen needle electrode, which is invasive and measures oxygen level at a single location.

We present our work on developing a combined treatment-imaging modality that integrates PDT and photoacoustic oxygen imaging. We propose a system designed for clinical treatments of cancer of the oral cavity. Tissue oxygen imaging is performed by applying Photoacoustic Lifetime Imaging (PALI). This technology relies on photoacoustic probing of oxygen-dependent excitation lifetime of Methylene Blue. The dye is excited by the same wavelength of illumination source for PDT. Once excited, the population of photosensitizer molecules at triplet state has a lifetime depending on the oxygen level. The transition from excited triplet state to ground state can be probe by another laser, which generate photoacoustic signal that is used to map the lifetime. The lifetime map is then converted to pO2 distribution.

We expect that PDT efficacy can be improved by applying PALI imaging feedback in real-time to determine, and individually optimize, O2-enriched gas breathing parameters and PDT light-dose during treatment. Successful implementation of PALI in PDT can also drive its application in guiding other cancer treatments that are affected by hypoxia.

8931-17, Session 4

Comparison of singlet oxygen threshold dose for PDT *(Invited Paper)*

Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States); Baochang Liu, Univ. of Pennsylvania (United States); Michele M. Kim, Univ. of Pennsylvania School of Medicine (United States); Dayton D. McMillan, Univ. of Pennsylvania (United States); Xing Liang, Jarod C. Finlay, The Univ. of Pennsylvania Health System (United States); Theresa M. Busch, Univ. of Pennsylvania (United States)

Macroscopic modeling of singlet oxygen (1O2) is of particular interest because it is the major cytotoxic agent causing biological effects for type II photosensitizers during PDT. We have developed a macroscopic model to calculate reacted singlet oxygen concentration ([1O2]rx for PDT. An in-vivo RIF tumor mouse model is used to correlate the necrosis depth to the calculation based on explicit PDT dosimetry of light fluence distribution, tissue optical properties, and photosensitizer concentrations. Inputs to the model include 4 photosensitizer specific photochemical parameters along with the apparent singlet oxygen threshold concentration. Photosensitizer specific model parameters are determined for several type II photosensitizers (Photofrin, BPD, and HPPH). The singlet oxygen threshold concentration is approximately 0.4 – 0.55 mM (or (3 – 5)x10^8 singlet oxygen per cell) for all three photosensitizers studied, assuming that the fraction of singlet oxygen generated that interacts with the cell is (f = 1). In comparison, values derived from in-vivo mice studies are 0.4 or 0.9 mM for mTHPC or Photofrin PDT, respectively. However, the singlet oxygen required per cell is reported 5x10^7 per cell per 1/e fractional kill in an in-vitro AML cell mTHPC-PDT study and is reported to be 12.1 mM for a multicell in-vitro EMT6/Ro spheroid model for Photofrin PDT. The sensitivity of threshold singlet oxygen dose for our experiment is examined. The possible influence of vascular vs. apoptotic cell killing mechanism on the singlet oxygen threshold dose will be discussed. The observed discrepancies between different experiments warrant further investigation to explain the cause of the difference.

8931-18, Session 4

High variability in patient-specific therapeutic dose observed during clinical aminolevulinic acid based photodynamic therapy treatments of actinic keratosis

Stephen C. Kanick, Scott C. Davis, Yan Zhao, Thayer School of Engineering at Dartmouth (United States); Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Edward V Maytin M.D., The Cleveland Clinic (United States); M. Shane Chapman M.D., Dartmouth Hitchcock Medical Ctr. (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States)

Actinic keratosis (AK) is a skin lesion that often progresses to squamous cell carcinoma. Photodynamic therapy (PDT) is a targeted treatment modality that is capable of inducing localized cell kill without causing lasting scarring, making it suitable for treating AK. Current clinical PDT treatments of AK involve topical administration of aminolevulinic acid (ALA), which is absorbed through the skin and induces preferential synthesis of the photosensiting agent protoporphryin IX (PpIX) in the irregularly functioning cells of the AK. While ALA-based PDT treatments have shown high clearance rates of AKs, a sizeable fraction of patients exhibit an incomplete response. The factors influencing efficacious PDT are complicated, but it is understood that sufficient PpIX in targeted lesions is required for effective treatment, and hypothetically, an approach to quantify PpIX could be used to guide patient-specific treatments. This study utilizes a fiber optic probe to monitor clinical treatments. Fluorescence measurements are used to quantify PpIX in AK lesions, and a paired white light reflectance measurement is used to characterize aspects of the local microvascular physiology (e.g. blood volume and hemoglobin saturation). Measurements are made prior to- and following administration of therapeutic light, allowing estimation of the patient-specific therapeutic dose delivered during treatment. Clinical observations show substantial inter-patient variability in PpIX fluorescence, which are correlated with metrics of physiological response (e.g. pain and skin reddening). This presentation will describe detailed aspects of the dosimetry system, incorporation of the measurements into the clinical treatment protocol, and preliminary results from an ongoing clinical investigation.

8931-19, Session 4

Recovery of optical properties from interstitial spectroscopy for photodynamic therapy treatment planning

Timothy M. Baran, Univ. of Rochester Medical Ctr. (United States); Michael C. Fenn, Univ. of Rochester (United States); Thomas H. Foster, Univ. of Rochester Medical Ctr. (United States)

Knowledge of optical properties is required to determine light dose in photodynamic therapy. We have designed an optical probe, consisting of six helically arranged side-firing fibers enclosed in a 1.1 mm diameter encapsulant, that can be used to determine these values. White light is delivered by one fiber, and detected by the others. Based on a Monte Carlo (MC) model of the probe, the absorption (μa) and reduced scattering (μs') coefficients of the sample are determined. Recovery was verified in tissue-simulating phantoms containing MnTPPS or intact human erythrocytes as absorbers, and Intralipid as scatterer. Mean errors in recovery of μa and μs' were 9% and 19%, respectively. In phantoms containing erythrocytes, hemoglobin oxygen saturation was recovered with mean error of 12%.

Using the MC model, we mapped the volumes sampled by particular spectroscopy fibers. For μa = 0.1 cm-1 and μs' = 20 cm-1, 49% of photon packets detected at the fiber adjacent to the source sampled a radius further than 5 mm from the probe, while 24% of photon packets sampled further than 7.5 mm. When μs' was reduced to 10 cm-1, 54% of photon packets traversed a radius greater than 5 mm from the probe and 29% sampled further than 7.5 mm. Changing the value of μa to 0.2 cm-1 did not have an effect on the sampled volume.

We also provide simulation results for a new probe design that aims to improve upon the accuracy of the current probe by incorporating a wider range of source-detector separations.

8931-20, Session 4

A fluorescence imaging system for sensitizer monitoring in pleural PDT

Jarod C. Finlay, Arash Darafsheh, Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States)

Dosimetry in intraoperative photodynamic therapy (PDT) is complicated by heterogeneity in optical properties, tissue oxygenation, and sensitizer distribution. Accounting for the heterogeneity in sensitizer distribution can be accomplished through fluorescence imaging at a resolution comparable to the scale of the heterogeneity. Quantitative imaging, however, requires disentanglement of the confounding effects of heterogeneous optical properties (absorption and scattering). We present the design and validation of a fluorescence imaging system for monitoring of sensitizer concentration during intraoperative PDT. The system consists of a structured-illumination system based on a miniature digital light projector (DLP), and an optical camera with various optical filters for spectral discrimination. We image each sample sequentially

using unmodulated white light, structure white light, and structured excitation light. The excitation light provides a fluorescence distribution image which is corrected for optical properties with an empirically calibrated correction derived from the structured white light image, and registered with the anatomical structures seen in the unmodulated white light image. We present an evaluation of the sensitivity and accuracy of fluorescence imaging based on numerical simulation and tests in tissue-simulating phantoms. The optical parameters of these models and phantoms are informed by previous measurements made in patients undergoing intraoperative PDT in the pleural cavity.

8931-21, Session 4

Multimodal imaging of skin cancer for treatment planning

Ulas Sunar, Daniel J. Rohrbach, Roswell Park Cancer Institute (United States); Daniel Muffoletto, Univ. at Buffalo (United States); Rolf B. Saager, Univ. of California, Irvine (United States); Kenneth Keymel, Anne Paquette, Janet Morgan, Joseph Housel, Natalie Zeitouni, Roswell Park Cancer Institute (United States)

PDT is an alternative treatment option for nonmelanoma skin cancers. PDT has shown lower success rates in treating thicker tumors. For effective PDT with minimal damage to normal tissue, an appropriate treatment field has to be outlined. Incorrect treatment planning may result in under-treatment and ultimately recurrence or over-treatment of surrounding normal tissue. Any tool that can help to delineate tumor would guide PDT and lead to better treatment planning.

Several noninvasive imaging modalities have been applied for skin cancer. Among them, high frequency ultrasound provides high resolution as well as deep penetration depth. However, the technique relies on mechanical contrast rather than functional contrast. Optical imaging can complement high resolution ultrasound with its high functional contrast and sensitivity. Spatial frequency domain imaging (SFDI) can provide optical (absorption and scattering), vascular (tissue oxygen saturation, blood volume) and fluorescence contrasts.

We will present preliminary results from our recent clinical trial. Our results indicates ultrasound can provide tumor thickness with high precision and SFDI provide enhanced contrast complementing the US scans. Thus multimodal approach can map optical and ultrasound contrasts to enable clinicians to better discriminate the tumors for treatment planning.

8931-22, Session 4

Optimization of the light profile in tissues for photodynamic therapy

Dilleys Ferreira da Silva, Thereza Cury Fortunato, Cristina Kurachi D.D.S., Vanderlei Salvador Bagnato, Univ. de São Paulo (Brazil)

A controlled illumination of lesions is a key ingredient for an efficient treatment by Photodynamic Therapy. Although this technique is very appropriate to surface lesions, its results are sometimes not as good as expected. Indeed, in 10 to 30% of skin cancer cases treated, the lesions are not completely destroyed. A homogeneous surface illumination may not be possible because shadow effects, slits or physical deformities on the lesion. Then, non-uniform illumination can result in partial necrosis regions, and then tumor recurrence. For this reason, it is crucial to improve the light profile inside the tissue.

We here show how to control the optical coupling, leading to a better tissue illumination and an efficient photosensitizer activation. The coupling is controlled by introducing a gel, between the light source (? = 630 nm) and the tissue, with low concentration of scatters. Our tests were conducted in vitro using a solid phantom, where the interface air/phantom mimics a rough tissue surface. Images of the propagating light beam within the phantom were collected and showed that the combination of gel and scatters leads to a strong improvement in the light distribution. In fact, a more homogeneous laser beam is delivered on the tissue, promoting an enhanced illumination and eliminating drastically the roughness effects of the sample exterior.

8931-23, Session 5

Novel indications for PDT of solid and cystic tumors of the pancreas *(Invited Paper)*

Stephen P. Pereira, Margaret G. Keane M.D., Stephen G. Bown M.D., Univ. College London (United Kingdom); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States); Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

Background: Pancreatic cancer (PanCa) is a devastating disease that is often diagnosed late and has few effective treatment strategies. Chemoradiotherapy has a limited survival benefit in locally advanced PanCa and is associated with significant systemic side effects. Additional diagnostic and treatment options are needed.

Methods and Results: Our phase I studies of interstitial PDT for locally advanced PanCa, showed that PDT can achieve a predictable volume of tumor necrosis with no serious adverse events. Following on from this work, we have developed a novel minimally invasive approach of endoscopic ultrasound-guided PDT (EUS-PDT) with online dosimetry for ablation of solid and cystic tumors of the pancreas. EUS-PDT individually and in combination with standard and novel chemotherapeutic agents could become an additional treatment option for this patient group.

Pancreatic cystic tumors are premalignant lesions of the pancreas which are an increasingly common clinical finding. At our tertiary referral center, 890 patients were diagnosed with pancreatic cystic lesions from January 2001 to September 2012. International guidance stipulates surveillance by non-invasive imaging and EUS for asymptomatic lesions <3cm. This practice is dramatically increasing hospital workloads and causing anxiety to patients. Endoscopic minimally invasive ablative treatments are an attractive alternative to surveillance. Our phase I studies in PanCa suggest that EUS-PDT has the potential to achieve complete ablation of small solid and cystic tumors of the pancreas with a favorable side effect profile.

Conclusion: EUS-PDT may significantly improve the length and/or quality of life of patients with pancreatic tumors, with significant cost-saving advantages.

8931-24, Session 5

Photodynamic therapy in the treatment of Barrett's esophagus: current status and future directions *(Invited Paper)*

Kenneth K. Wang M.D., Mayo Clinic (United States)

Barrett's esophagus is a pre-malignant tissue that has been the only known precursor for the treatment of Barrett's esophagus. Its treatment with phototherapy was one of the earliest approved indications in the United States. However, the mechanism of ablation is still unclear and the source of the regenerated tissue has not been determined. We will discuss results of creating Barrett's esophagus with a novel IL-1B L2 mouse with chronic inflammation of the foregut. The importance of senescence in response to Barrett's esophagus and how this interacts with the IL6 signaling pathway. The results of both in vivo and ex vivo studies will be presented. The role of p16 in this process will be discussed as well. In addition, there appear to be biological features that make ablative therapy very difficult to implement such as the length of the Barrett's segment. This will be presented with the results of phototherapy clinical trials. Neosquamous repopulation has been difficult to predict.

8931-25, Session 5

STAT3 Expression Reduces PDT Efficacy in Malignant Pleural Mesothelioma: combined analysis of clinical tissue microarrays and preclinical 3D tumor nodules with inducible shRNA knockdown *(Invited Paper)*

Keith A. Cengel, Univ. of Pennsylvania School of Medicine (United States)

Patients with locally advanced mesothelioma (MPM) treated with radical pleurectomy (RP) and pleural photodynamic therapy (pPDT) demonstrate an unprecedented median survival of over 41 months. However, with a median follow-up of 31 months, 61% (41/67 patients) experience a local relapse(LR) and patients who experience an early recurrence (< median,11.6mo) demonstrate a significantly decreased median survival (earlyLR: 10.8mo vs lateLR: 54.7mo, p<0.0001]. Immunohistochemistry(IHC) of tissue microarrays demonstrate that this disparity is associated with differences in tumor expression of STAT3 (normalized staining index Early LR 82/100 vs LateLR 62/100 p<0.001). To evaluate the causal nature of the relationship between molecular phenotype and PDT efficacy, we developed a 3D tumor nodule system using human MPM cells that have doxycycline inducible expression of either non-silencing or STAT3 specific shRNA. The presence of the uninduced vector or a 4 day induction of shRNA expression (either shNS or shSTAT3) in nodules grown for 8-10 days prior to induction do not affect nodule growth rate and morphology and STAT3 knockdown in these nodule has been confirmed both by both IHC/confocal microscopy and western blotting. Preliminary studies using this 3D nodule system show decreased apoptosis at 24h following PDT of STAT3 expressing vs non-expressing nodules. Moreover, standard monolayer based clonogenic survival studies using REN shNS and shSTAT3 show that STAT3 expression significantly reduces PDT-induced direct cell cytotoxicity. Taken together, these data suggest that STAT3 expression leads to decreased local control in patients with MPM by a mechanism that involves STAT3-mediated resistance to PDT-induced apoptosis.

8931-26, Session 5

Receptor concentration imaging (RCI) can quantify available epidermal growth factor status after photodynamic therapy in pancreatic cancer

Kimberley S. Samkoe, Geisel School of Medicine at Dartmouth (United States) and Thayer School of Engineering at Darmtouth College (United States); Kenneth M. Tichauer, Illinois Institute of Technology (United States); Jason R. Gunn, Thayer School of Engineering at Dartmouth (United States); Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States) and Geisel School of Medicine at Dartmouth (United States)

Verteporfin photodynamic therapy (PDT) for the treatment of pancreas cancer (PCa) is showing promise as a therapy for patients with unresectable tumors. Measured changes in tumor volume after PDT are the conventional indicator for tumor status post-therapy; however, if the tumor is imaged too close to the time of therapy these changes can be more reflective of hemodynamics, edema and inflammation. A previous magnetic resonance (MR) imaging study performed by our group indicated that PDT effect can be observed 48 hrs post-PDT but large amounts of inflammation and edema may confound results. Alternative approaches to monitoring tumor status exploit these changes by monitoring the overexpression of tumor-specific cell-signaling receptors, such as fluorescently labeled epidermal growth factor receptor (EGFR), and can be performed at much earlier time points post-therapy. Unfortunately, these techniques can be skewed by the enhanced permeability and retention (EPR) effect as well as hemodynamics and inflammation as in MR imaging. We have recently demonstrated that Receptor Concentration Imaging (RCI), a method of quantifying cell-signaling receptors, is independent of these confounding factors by monitoring the kinetics of a targeted and untargeted pair of imaging agents instead of monitoring total agent accumulation. Here, we will use MR imaging, fluorescence contrast imaging and RCI using a murine xenograft orthotopic PaC to measure tumor response to interstitial verteporfin PDT (1mg/kg, 20J/cm) at 1, 3 and 7 days post-therapy. RCI has the capability to provide immediate quantification of the tumor molecular response to therapy and the potential of guiding early subsequent molecular-based adjuvant therapies.

8931-27, Session 5

Photodynamic inactivation of pathogens causing infectious keratitis

Carole Simon, Technische Univ. Kaiserslautern (Germany); Georg Wolf, Department of Medical Physics, Technical University of Kaiserslautern (Germany); Meik Walther, Technische Univ. Kaiserslautern (Germany); Katrin Winkler, Melanie Finke, Univ. des Saarlandes (Germany); Dirk Hüttenberger, ApoCare Pharma GmbH (Germany); Markus Bischoff, Berthold Seitz, Univ. des Saarlandes (Germany); John Cullum, Hans-Jochen Foth, Technische Univ. Kaiserslautern (Germany)

The increasing prevalence of antibiotic resistance requires new approaches for the treatment of infectious keratitis. Photodynamic Inactivation (PDI) using the photosensitizer (PS) chlorin e6 (Ce6) was investigated as an alternative to antibiotic treatment. An in-vitro cornea model was established using porcine eyes. The uptake of Ce6 by bacteria and the diffusion of the PS in the individual layers of corneal tissue were investigated by fluorescence. After removal of the cornea's epithelium Ce6-concentrations < 1 mM were sufficient to reach a penetration depth of 500 µm. Liquid cultures of microorganisms were irradiated using a specially constructed illumination chamber made of Spectralon® (reflectance: 99%), which was equipped with high power light emitting diodes (?=670 nm). Clinical isolates of Staphylococcus aureus (SA) and Pseudomonas aeruginosa (PA) from keratitis patients were tested in liquid culture against different concentrations of Ce6 (1-512 µM) using 10 minutes irradiation (E=18 J/cm?). This demonstrated that a complete inactivation of the pathogen strains was feasible whereby SA was slightly more susceptible than PA. 3909 mutants of the Keio collection of Escherichia coli were screened for potential resistance factors. The sensitive mutants can be grouped into three categories: transport mutants, mutants in lipopolysaccharide synthesis and mutants in the bacterial SOS-response. In conclusion PDI is seen as a promising therapy concept for infectious keratitis.

8931-28, Session 5

Photodynamic effect of photosensitizer-loaded hollow silica nanoparticles for hepatobiliary malignancies: an in vitro and in vivo study

Xiaofeng Deng, Li Xiong, Yu Wen, Zhongtao Liu, Dongni Pei, Yaxun Huang, Xiongying Miao, The Second Xiangya Hospital (China)

Background and aims: Nanoparticles have been explored recently as an efficient delivery system for photosensitizers in photodynamic

therapy. In this study, polyhematoporphyrin (C34H38N4NaO5,) was loaded into hollow silica nanoparticles (HSNPs) by one-step wet chemical-based synthetic route. We evaluate the efficacy and safety of polyhematoporphyrin-loaded HSNPs with hepatobiliary malignant cells and in vivo models.

Methods: Human liver cancer, cholangiocarcinoma and gallbladder cancer cells were cultured with the HSNPs and cellular viability was determined by MTT assay. Apoptotic and necrotic cells were measured by flow cytometry. Finally, we investigate its effect in vivo.

Results: In MTT assay, the cell viability of HepG-2, Huh-7, QBC939 and GBC-SD cells of the HSNPs was 4.7±2.0%, 6.5±1.2%, 6.4±1.3%, 3.7±1.2% respectively, which were significant different from that of free polyhematoporphyrin 44.3±1.9%, 62.5±6.0%, 62.4±4.7%, 33.4±6.5%. Flow cytometry demonstrated the laser-induced cell death with polyhematoporphyrin-loaded HSNPs was much more severe. Similarly, in vivo results of each kind of cell revealed 14 days post-photoradiated, tumor sizes of the HSNPs group were significantly smaller. Administration of the HSNPs without illumination cannot cause killing effect both in vitro and in vivo experiments.

Conclusions: HSNPs is a desirable delivery system in photodynamic therapy for hepatobiliary malignacies, with improved aqueous solubility, stability and transport efficiency of photosensitizers.

8931-29, Session 6

Prospects for future adoption of photodynamic therapy as a mainstream treatment of nonmelanoma skin cancer in the USA *(Invited Paper)*

Edward V. Maytin M.D., The Cleveland Clinic (United States)

Nonmelanoma skin cancers (NMSC), comprising basal cell (BCC) and squamous cell carcinoma (SCC), are the most common of all human malignancies (10x more prevalent than breast cancer), and represent a major healthcare major burden. Various destructive modalities including surgical excision are widely available for NMSC, but all cause scarring and disfigurement. Photodynamic therapy (PDT) with topical aminolevulinic acid (ALA) is a non-scarring modality with good efficacy for NMSC, convincingly shown by clinical trials in Europe where PDT is now licensed for NMSC. In the U.S.A. however, ALA-PDT is FDA-approved only for actinic keratoses. Reasons include a need for more standardization, clinical studies, and proper alignment of market forces. However, PDT already offers great promise as an alternative to surgery in several high-risk subpopulations: (1) SCC in organ transplant patients; (2) SCC in Fitzpatrick Type 1 photodamaged patients; (3) BCC in basal cell nevus syndrome (BCNS; Gorlin's). In these groups, new tumors arise so frequently that surgery often cannot keep up, despite extensive scarring. ALA-PDT on the other hand can be given repeatedly while offering scarless healing and cancer-prevention benefits. We will review two approaches currently being explored to boost efficacy: (1) cyclic treatment regimens; (2) combination approaches. The rationale for cyclic regimens is that although PDT may not clear tumors completely after one session, PDT can be repeated indefinitely because it is non-mutagenic. As a combination approach, 5-Fluorouracil is given prior to ALA to boost intratumoral photosensitizer concentrations and pro-apoptotic mechanisms; supportive evidence from our ongoing clinical trial will be presented.

8931-30, Session 6

To be announced *(Invited Paper)*

Merrill A. Biel M.D., Univ. of Minnesota (United States)

No Abstract Available

8931-31, Session 6

Pulsed light imaging for wide-field dosimetry of photodynamic therapy in the skin

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Actinic Keratoses (AK) are pre-cancerous skin lesions which are non-life-threatening for the general population but pose a significant risk for organ transplant recipients (OTR) undergoing immunosuppressant therapy. These patients can suffer widespread and recurrent AK leading to aggressive malignant squamous cell carcinoma at alarming rates. While there are several treatment modalities available for AK, ALA-based photodynamic therapy is particularly attractive for OTR's since it can be applied to wide areas of the skin and repeated frequently; however, response rates vary widely, and a sizeable percentage of lesions have no response. This is predominantly due to lack of photosensitizer accumulation in the lesions, which mitigates the effect of the light dose; however, there are currently no clinical tools to determine drug uptake before light delivery to facilitate patient-specific treatment parameters. To address this need, we have developed a novel wide-field imaging system based on pulsed excitation and gated acquisition to image photosensitizer activity. The tissue is illuminated using four pulsed LED's to excite PpIX, and the remitted light acquired with a synchronized ICCD. This approach facilitates real-time background subtraction of ambient light, precluding the need to darken the exam room. Delivering light in short bursts also allows the use of elevated excitation intensity while remaining under the maximum permissible exposure limits, making the modality more sensitive to photosensitizer fluorescence than standard approaches. Images of tissue phantoms indicate system sensitivity down to 250nM PpIX and images of animals and humans demonstrate detection of PpIX fluorescence in vivo under normal room light conditions.

8931-32, Session 6

Photodynamic therapy of cervical intraepithelial neoplasia

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More than 90% of cervical cancers originate in the evolution of cervical intraepithelial neoplasia (CIN). CIN may be categorized into grades I, II and III depending upon the proportion of the thickness of the epithelium showing mature and differentiated cells. Cervical cancer is the third most common cancer and the fourth leading cause of death in women worldwide. In 2008, an estimated 529,800 new cases were diagnosed and 275,100 deaths occurred due to cervical cancer worldwide. Eighty six percent of these cases occur in developing countries, representing 13% of all female cancers. In Brazil alone, current estimates indicate that every year 18,430 women are newly diagnosed with cervical cancer and about 4,800 die from the disease. Photodynamic therapy (PDT) is a therapeutic modality that has great potential to increase the resolution of

clinical diagnosis of CIN I, II and III and less invasive than conventional treatment. This is a controlled randomized clinical trial of photodynamic diagnostic and treatment for cervical intraepithelial neoplasia in women with a positive cytology (Pap test) confirmed by a positive cervical biopsy (grades CIN I, II and III). The prodrug containing 20% (w/w) methyl aminolevulinate (PDTPharma, SP-Brazil) is applied topically inducing protoporphyrin IX (PpIX) production. Fluorescence images of PpIX are obtained one hour after with a laser emitting at 400 nm, and another tip with LEDs (light-emitting diodes) at 630 nm is anatomically positioned covering the entire cervix. The equipment used in this study is the prototype "CERCA" produced by MMOptics (São Carlos, SP, Brazil).

8931-33, Session 6

Definitive surgery and intraoperative photodynamic therapy: a prospective study of local control and survival for patients with pleural dissemination of non-small cell lung cancer

Charles B. Simone M.D., The Univ. of Pennsylvania Health System (United States); Keith A. Cengel, Univ. of Pennsylvania School of Medicine (United States)

Definitive surgery typically has no role in the treatment of patients with Non-small cell lung cancer (NSCLC) with pleural spread, with studies showing up to 90% local relapse rates and no increase in the 6-9 month median overall survival (OS) observed with palliative chemotherapy. Here, we report clinical results in 34 consecutive patients enrolled between 1997-2012 on IRB-approved prospective clinical trials definitive surgery (gross total resection) with pleural photodynamic therapy (pPDT). The cohort was 50% male, predominantly Caucasian(85%), and a median of 55yrs at the time of surgery (range,35-73yrs). Over half(56%) underwent pneumonectomy, whereas 38% received radical pleurectomy . Pathologic staging was pT4N0 (24%) or pT4N2 (76%) and postoperative radiotherapy to the mediastinum or chemotherapy was used in 59% and 50% of patients, respectively. Pleural recurrence rates and OS were similar for patients undergoing pneumonectomy or other procedures (p>0.05 for both). Cohort median OS was 21.4 months (0.4-161.1 months), and survival rates were 59% at 1yr and 41% at 2yrs. Median overall PFS was 7.5 months, with numerous patients achieving durable disease-free intervals (mean PFS 18.4 months). Median pleural PFS was 13.6 months, with pleural recurrences occurring in only 32% of patients. In conclusion, this study demonstrates that surgery and intraoperative PDT can achieve durable local control and prolonged survival for NSCLC patients with pleural dissemination. Compared with current standard treatment, which offers a median survival of 6-9 months from pleural metastasis diagnosis, our cohort lived a median of 24.7 months from pleural diagnosis and 21.4 months from surgery/PDT.

8931-34, Session 7

Porphyrin-based polysilsesquioxane nanoparticles to improve photodynamic therapy for cancer treatment

Juan L. Vivero-Escoto, Daniel DeCillis, Laura Fritts, The Univ. of North Carolina at Charlotte (United States); Daniel L. Vega, The Univ. of North Carolina at Charlotte (United States) and The Center for Biomedical Engineering and Science, University 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 (e.g. 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 (PSQ) nanoparticles are crosslinked homopolymers formed by the condensation of functionalized trialkoxysilanes or bis(trialkoxysilanes). We believe that PSQ particles provide an interesting platform for developing PS 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, PSQ 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 can be further functionalized with polyethylene glycol (PEG) and targeting ligands to improve its biocompatibility and target specificity. The effectiveness of this porphyrin-based hybrid nanomaterial was successfully demonstrated in vitro using human cervical and pancreatic cancer cells.

8931-35, Session 7

Diffuse optical tomography using multichannel robotic platform for interstitial PDT

Anna V. Sharikova, Xing Liang, Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States)

In the operating room, time is extremely precious, and the speed of one's system often determines whether the data will be taken or not. Our multichannel robotic platform addresses this issue by optimizing source and detector scanning procedures. Up to 16 fibers were moved independently with resolution of 0.05 mm and speed of 50 mm/s using motors with position feedback. The initial fiber alignment employed a light beam/optical detector system for identical positioning of all motors. Peak and edge detection algorithms, for point and linear sources, were used with multiple fibers simultaneously for fast realignment of sources and detectors. The robotic platform was then used to perform Diffuse Optical Tomography (DOT) measurements in solid prostate phantoms with both homogenous and inhomogeneous Optical Properties (OP). Correct positioning was critical for the accurate recovery of the OP. The light fluence rate distribution was determined by scanning multiple detector fibers simultaneously along groups of lit linear sources placed throughout the phantom volume inside catheter needles. The scanning time for the entire DOT was about 10 seconds after alignment. The OP distribution reconstruction was based on the steady-state light diffusion equation. The inverse interstitial DOT problem was solved using NIRFAST. The optical properties were recovered by iterative minimization of the difference between measured and calculated light fluence rates. Recovered OP agreed with the actual values within 10%. The OP corrections were used to dramatically improve light fluence accuracy for the entire volume of bulk tumor.

8931-36, Session 7

Perfusion CT as a surrogate for verteporfin PDT dosimetry in pancreatic cancer: validation in rabbit tumor model studies

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Institute of Technology (United States); Karen L. Moodie, Susan Kane, Geisel School of Medicine at Dartmouth (United States); P. Jack Hoopes, Geisel School of Medicine (United States); Ting-Yim Lee, Robarts Research Institute (Canada); Stephen P. Pereira, Univ. College London (United Kingdom); Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Brian W. Pogue, Thayer School of Engineering at Dartmouth (United States) and Dartmouth Hitchcock Medical Ctr. (United States)

Interstitial photodynamic therapy (PDT) is being investigated in an ongoing clinical trial for the treatment of pancreatic cancer. The locations for fiber placement are pre-determined using X-ray computed tomography (CT) imaging and are physically placed under endoscopic ultrasound (US) guidance. In vivo light dosimetry has been attempted through these fiber ports using single measurements of localized drug concentration; however, accurate and representative measurements of photosensitizer uptake in the tumor is difficult due to the high variation in drug concentrations and the complexity and location of the disease. Using a rabbit model of pancreatic cancer (VX2 tumor line), we hypothesize that perfusion CT scans (using a whole body multi-slice CT scanner designed for clinic) of pancreatic tumors can accurately predict the photosensitizer delivery during PDT treatment. Contrast enhanced CT imaging was performed 7-days post-implantation to determine tumor size and location. At 10-days post-implantation, perfusion CT scans were performed using OmnipaqueTM (GE Healthcare) contrast agent. Post-CT imaging, verteporfin (1 mg/kg) was administered intravenously, allowed to distribute for one hour and sliced into sections correlating to each CT image slice. Spatial verteporfin uptake and distribution in fluorescent images were compared to the spatial hemodynamic parameters determined by CT (blood flow, blood volume, mean transit time and permeability surface area product). Correlation of the CT to verteporfin fluorescence could provide a surrogate measure for clinical PDT treatment planning in future work.

8931-37, Session 7

Association of optical clarity and photodynamic therapy for melanoma: in vivo study at tumor model

Layla Pires, Lilian Tan Moriyama, Clóvis Grecco, Sebastião Pratavieira, Cristina Kurachi D.D.S., Univ. de São Paulo (Brazil)

Photodynamic therapy (PDT) is a technique used for cancer treatment, mainly for non-melanoma skin cancer (nMSC). Although nMSC is the most prevalent type, melanoma is responsible for approximately 85% of deaths caused by skin cancer. Optical characteristics of the melanoma make its treatment with PDT a huge challenge. Studies involving PDT and melanoma are mainly based on the evaluation of new photosensitizers that absorb light in the infrared region, but complete response was not observed for most of the cases. In this study, the association of tissue optical clarity and PDT was evaluated in an in vivo murine melanoma model. Melanoma cells (B16F10) were injected intradermically in nude mice and treatment was performed when the tumor size reached 1cm2. The optical clarity solutions were topically applied at the lesion, after a tape stripping process. The total light attenuation coefficient was measured by reflectance spectroscopy. Preliminary results show that the total attenuation coefficient in normal skin decreases in approximately 14% and for melanoma, the decrease ranged from 60 to 92%. Biological features were evaluated by histology and confocal microscopy. After clarity, melanoma was treated using chlorine or porphyrin, intravenously injected, and illumination at 660 nm and 630 nm, respectively. Photosensitizer kinetic studies were also performed and indicated that chlorines are more selective than porphyrin compounds. These preliminary results show that the use of optical clarity solutions and chlorine as a photosenstizer may improve the PDT effect on melanoma. Financial support: Capes.

8931-38, Session 8

Investigation of dynamic morphological changes of cancer cells during photoimmuno therapy (PIT) by low-coherence quantitative phase microscopy

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We have reported a new molecular-targeted cancer photo-therapy, photoimmunotherapy (PIT), which completely cured tumors in mice without any side-effects. To understand the mechanism of PIT, dynamic morphological changes of cancer cells during PIT were investigated by a low-coherence quantitative phase microscope (LC-QPM) developed by Hamamatsu Photonics K.K..

3T3/HER2 cells were incubated with anti-HER2 trastuzumab-IR700 (10 ?g/mL, 0.1 ?M as IR700) for 24 hours, then, three-dimensionally imaged with the LC-QPM during the exposure of two different optically filtered lights for excitation of IR700 (500-780 nm) and imaging (780-950 nm). For comparison with traditional PDT, the same experiments were performed with Photofrin (10 and 1 ?M).

Serial changes on the cell membrane were visualized on 3D dynamic views. 3T3/HER2 cells began to swell rapidly after exposure to 500-780 nm light excitation. The cell volume reached a maximum within 1 min after continuous exposure, and then cells were deflated. This finding suggests that PIT damages the cell membrane by photo-reaction inducing influx of water leading to swelling and bursting of the cells. Interestingly, slow swelling and delayed burst of cells were observed even after only 5-sec exposure of excitation light, thus sufficient cumulative damages on the cell membrane induce lethal damage to cells. Similar, non-selective membrane damage was detected in Photofrin-treated cells.

Thus, PIT induces sufficient cumulative damage to the cell membrane within 5 seconds to induce rapid necrotic cell death which can be observed directly with LC-QPM. Further investigation is needed to evaluate the biochemical mechanisms underlying PIT-induced cellular membrane damage.

8931-39, Session 8

ALA-PpIX variability quantitatively imaged in A431 epidermoid tumors using in vivo ultrasound fluorescence tomography and ex vivo assay

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Treatment monitoring of Aminolevunilic-acid (ALA) - Photodynamic Therapy (PDT) of basal-cell carcinoma (BCC) calls for superficial and subsurface imaging techniques. While superficial imagers exist for this purpose, their ability to assess PpIX levels in thick lesions is poor; additionally few treatment centers have the capability to measure ALA-induced PpIX production. An area of active research is to improve treatments to deeper and nodular BCCs, because treatment is least effective in these.

The goal of this work was to understand the logistics and technical capabilities to quantify PpIX at depths over 1mm, using a novel hybrid ultrasound-guided, fiber-based fluorescence molecular spectroscopic-tomography system. This system utilizes a 633nm excitation laser and detection using filtered spectrometers. Source and detection fibers are collinear so that their imaging plane matches that of ultrasound transducer. Validation with phantoms and tumor-simulating fluorescent inclusions in mice showed sensitivity to fluorophore concentrations as low as 0.025µg/ml at 4mm depth from surface, as presented in previous years.

Image-guided quantification of ALA-induced PpIX production was completed in subcutaneous xenograft epidermoid cancer tumor model A431 in nude mice. A total of 32 animals were imaged in-vivo, using several time points, including pre-ALA, 4-hours post-ALA, and 24-hours post-ALA administration. On average, PpIX production in tumors increased by over 10-fold, 4-hours post-ALA. Statistical analysis of PpIX fluorescence showed significant difference among all groups; $p<0.05$. Results were validated by ex-vivo imaging of resected tumors. Details of imaging, analysis and results will be presented to illustrate variability and the potential for imaging these values at depth.

8931-40, Session 8

In vitro studies of chlorin e6-assisted photodynamic inactivation of Helicobacter pylori

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Helicobacter pylori (HP), a gram-negative microaerophilic bacterium located in gastric mucosa, plays an important role in gastro carcinogenesis. Due to the increasing emergence of antibiotic resistance, photodynamic inactivation of bacteria presents a new approach to treat chronic bacterial stomach infections. In vitro experiments were performed to determine the irradiation conditions for a complete inactivation of HP with the photosensitizer chlorin e6 (Ce6). The HP strain CCUG 38770 (Culture Collection, University of Götenburg, Sweden) was routinely cultured under microaerophilic conditions, suspended in sodium chloride, incubated with Ce6 and irradiated briefly with red light appropriate wavelength (?=660 nm). Series of measurements of different concentrations (0.1 µM - 100 µM) were carried out, whereby the incubation time was kept constant at 1 min. The absorbed energy dose has been set in varying the irradiation time (1 s - 300 s) and the power density (4.5 mW/cm? - 31 mW/cm?). Quantification of inactivation was performed by enumeration of the grown colonies. In addition, the accumulation of Ce6 in HP cells was studied more precisely by fluorescence spectroscopy. With a Ce6 concentration of 100 µM and a power density of 9 mW/cm?, a 6-log reduction in the survival rate of HP was achieved within 30 seconds of radiation. In conclusion the most relevant factor for the inactivation of HP is the exposure time of irradiation, followed by the concentration of Ce6 and the light intensity. Further studies with HP strains obtained from patient specimens are under current investigation.

8931-41, Session PMon

Optimization of topical PDT using ALA and metil-ALA mixtures evaluated by fluorescence spectroscopy and widefield fluorescence imaging

Priscila Fernanda Campos Menezes, Michelle B. Requena, Alessandra K. K. Fujita, Cristina Kurachi D.D.S., Univ. de São Paulo (Brazil); Andrigo B. de Nardi, Andre Escobar, Univ. Estadual Paulista (Brazil); Vanderlei Salvador Bagnato, Univ. de São Paulo (Brazil)

The PDT using ALA and Methyl ALA (M-ALA) have been used in the treatment of different types of cancer. In the topic application using ALA or M-ALA as precursor of PPIX, there are many limitations. The goal of study was determine the best mixture using ALA and M-ALA, to be applied in PDT, by optical permeation methods. This study was performed in vivo, using porcine and human skin models. The cream was applied at 6cm2 and an occlusive dressing placed. The seven mixtures (M1-M7) used are described respectively as: 100% ALA-0% M-ALA; 80% ALA-20% M-ALA; 60% ALA-40% M-ALA; 40% ALA-60% M-ALA; 20% ALA-80% M-ALA; 0% ALA-100% M-ALA. The PpIX production was monitored using widefield fluorescence imaging and fluorescence spectroscopy collected at skin surface to each hour, until 5h of treatment. The results obtained with porcine skin using widefield fluorescence imaging, show that, the PPIX production, at superficial layers, is greater and more homogeneous using around 40-60% of ALA mixtures. Also, the same results with human skin were observed. The PPIX formation in deeper layers was greater and faster to mixture of 100% of ALA. However the emulsion with 50% of ALA and M-ALA mixture increases the PPIX production after 5h in deeper layers. The mixture rich in M-ALA for both layers showed lower PPIX production. In this study we prove the similarity of skin models by optical permeation techniques. These results can be useful in Clinical Applications of PDT using topical ALA and M-ALA (Financial support: FAPESP).

8931-42, Session PMon

Singlet oxygen-based dosimetry for BPD-PDT efficacy

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The emerging and expanded use of photodynamic therapy (PDT) as a cancer treatment option offers a unique approach to treat and control localized tumors. Though new PDT protocols are being used, accurate dosimetry remains a significant challenge. The ability to efficiently predict how established tumors can be controlled by a given PDT treatment is often limited using current PDT dosimetric methods such as total light fluence and PDT dose, as these methods may not account for photosensitizer concentration, light fluence rate, or oxygen depletion variables. We aim to improve upon PDT dosimetry by showing correlation reacted singlet oxygen concentration ([1O2]rx), the product of a previously established macroscopic explicit dosimetry model, and tumor control is superior to other dosimetric methods. Tumored mice were treated under a variety of light fluence and fluence rate conditions and measurements were taken to quantify total light fluence, PDT dose, and [1O2]rx. Changes in tumor volume were tracked following treatment and compared with the three calculated dose metrics. The comparison of these dose metrics shows [1O2]rx serves as a useful dosimetric quantity for predicting treatment outcome for a clinically relevant endpoint in tumor growth.

8931-43, Session PMon

Anisotropic modeling for IR navigation-based PDT dosimetry

Michele M. Kim, Univ. of Pennsylvania School of Medicine (United States); Xing Liang, Univ. of Pennsylvania (United States); Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States)

An infrared (IR) camera system has been developed for use in pleural photodynamic therapy (PDT). This system was introduced to pleural PDT to provide uniform light dose distribution to ensure predictable PDT outcome. Light is delivered through a fiber that is in an endotracheal (ET) tube filled with Intralipid as scattering media. A tracking tool is attached to the ET tube to monitor the position of the optical fiber based point source. An anisotropic light distribution model is introduced to correct the angle dependent light distribution due to a capped end by design of the ET tube, which scatters light differently than the sides. In this study, the anisotropic nature of the balloon was characterized and incorporated into the calculation for light fluence during treatment. This model is verified by the light dose calculation from a phantom study. Furthermore, a new tracking tool was designed with multiple faces to increase the angular field of view and thus collect more viable data during treatment. The new tracking tool is directly entered into the ET tube with the light delivering fiber, thus eliminating the need to calibrate the laser source position prior to treatment via an optimization method. With this improved system, the calculated light fluence and the measured isotropic detector readings are more accurately matched.

8931-44, Session PMon

Photodynamic and biological activity of new cationic porphyrins in vitro

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We synthesized a set of water-soluble cationic porphyrins (PSs) with different peripheral functional groups and metalloporphyrins with different central metal atoms that were found be good candidates for PDT and photodynamic inactivation of microorganisms. Cancer cell ?ulture (monolayer and suspension) equally well destroyed by cytotoxic or phototoxic action of new cationic porphyrins with charge +3 and +4. By photodynamic effect on culture of suspension cells K-562 (chronic myelogenous leukemia lymphoblasts) the most effective preparations are Zn-metalloporphyrins with hydrocarbon “tails” (-C12 or -C16). Photodynamic actions of metalloporphyrins are in 10-20 times more effective than the cytotoxic action the same porphyrins. It is established that in vitro for the destruction of Gram (+) and Gram (-) microorganisms in photodynamic mode cationic water-soluble synthetic metalloporphyrins, especially Zn-meso-tetra-[4-N-(2`- butyl) pyridyl] porphyrin (Zn-TBut4PyP), many times more effective than Zn-pheophytins, synthesized from the nature origin. In vivo conditions on mice established that the best therapeutic activity against various strains of the microorganism St. aureus has the synthetic metalloporphyrin Ag-TBut4PyP. For PDT among blood proteins the most important in the transport of porphyrins are serum albumin, lipoproteins, and hemoglobin. Via different methods of optical spectroscopy it was found, that long-chain fatty acids, palmitic and stearic acids, compete with cationic porphyrins for binding to the heme high-affinity site of human serum albumin (HSA). Computer simulation (docking) of cationic porphyrins into the subdomain I B of HSA revealed the amino acids that differentially interact with peripheral functional groups of PSs and thereby, could affect the affinity for HSA.

8931-45, Session PMon

Evaluation of protoporphyrin ix production using different mixtures of ALA and M-ALA by widefield fluorescence in porcine skin model

Alessandra K. K. Fujita, Univ. de São Paulo (Brasil); Priscila Fernanda Campos Menezes, Michelle B. Requena, Cristina Kurachi D.D.S., Vanderlei Salvador Bagnato, Univ. de São Paulo (Brazil)

Topical PDT using ALA (aminolevulinic acid) or methyl-ALA (MAL) as precursor of protoporphyrin IX (PpIX) shows some limitations. ALA is more hydrophilic than MAL, resulting in differences at the molecule interaction with the cells. The goal of study is evaluated the optimization of topical PDT using ALA and Metil-ALA in different mixtures. This study was done in vivo, using porcine skin models using 7 categories of ALA and M-ALA mixtures: 100%ALA- 0%M-ALA; 80%ALA- 20%M-ALA; 60%ALA- 40%M-ALA; 40%ALA- 60%M-ALA; 20%ALA- 80%M-ALA; 0%ALA- 100%M-ALA. The cream in different mixtures was applied at 4cm2 and an occlusive dressing placed. PpIX production in a porcine skin model was monitored using widefield fluorescence imaging (400-450 nm excitation) and fluorescence spectroscopy (532 nm excitation) collected at skin surface during 5h of treatment. Seven types of mixture were tested, from 0-100% combinations, varying on 20%. The imaging analyses, showed the best results in PpIX production with mixtures from 40% to 80% of ALA. Both fluorescence techniques showed that the higher relative production of PpIX was present when the ALA concentration was of 40-80%. Widefield fluorescence imaging showed a more homogenous PpIX production at the skin surface for the higher ALA concentrations. The investigation of the PpIX production as a function of the tissue depth is being performed. These results can be useful in Clinical Applications of PDT using topical ALA and M-ALA (Financial support: CNPq).

8931-46, Session PMon

Blood flow and oxygenation correlate with local PDT dose and response

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Noninvasive optical spectroscopy can provide near real-time feedback on parameters related to therapy efficacy. Here we tested whether pre-treatment and early changes in blood flow and oxygenation will be indicative of PDT response in an animal and a clinical study.

C3H mice with subcutaneous SCCVII tumors were treated with 0.47 µmol/kg HPPH and 100 J but at two fluence rates: low fluence rate (14mW/cm2) and high fluence rate (75mW/cm2). Tumor blood flow was monitored continuously during PDT using diffuse correlation spectroscopy (DCS). After treatment the tumors were removed to determine the percent crosslinking of Signal Transducer and Activator of Transcription 3 (STAT3), a molecular biomarker for photoreaction. We observed significant differences in blood flow changes between the two fluence rate groups. Blood flow showed a high correlation with STAT3 crosslinking (r2 = 0.87). Mice in the low fluence rate group showed higher average STAT3 crosslinking than those in the high fluence rate group (19.2 ± 6.1% vs. 7.0 ± 2.8% respectively). Continuous monitoring of blood flow changes can provide an in vivo marker related to local PDT dose that is indicative of PDT efficacy. Providing real-time feedback can also lead to patient specific oxygen conserving regimens and more effective PDT.

We further investigated these parameters in clinical-PDT for head and neck cancer of the oral cavity. Preliminary results indicate that blood flow correlates with STAT3 crosslinking while the combination of multiple parameters including blood oxygenation, blood flow and photosensitizer content is the strongest predictor of response.

8931-47, Session PMon

Comparison of PDT parameters for RIF and H460 tumor models during HPPH-mediated PDT

Baochang Liu, Univ. of Pennsylvania (United States); Michele M. Kim, Univ. of Pennsylvania School of Medicine (United States); Shannon M. Gallagher-Colombo, Theresa M. Busch, Univ. of Pennsylvania (United States); Timothy C. Zhu, The Univ. of Pennsylvania Health System (United States)

Singlet oxygen (1O2) is the major cytotoxic species producing PDT effects, but it is difficult to monitor in vivo due to its short life time in real biological environments. Mathematical models are then useful to calculate 1O2 concentrations for PDT dosimetry. Our previously introduced macroscopic model has four PDT parameters: ?, ?, ?, and g, describing initial oxygen consumption rate, ratio of photobleaching to reaction between 1O2 and cellular targets, ratio of triplet state (T) phosphorescence to reaction between T and oxygen (3O2), and oxygen supply rate to tissue, respectively. In addition, the model calculates a fifth parameter - threshold 1O2 dose ([1O2]rx). These PDT parameters have been investigated for HPPH using radiation-induced fibrosarcoma (RIF) tumors in an in-vivo mouse model. In recent studies, we additionally investigate these parameters in human non-small cell lung carcinoma (H460) tumor xenografts, also using HPPH-mediated PDT. In-vivo studies are performed of C3H female mice with H460 tumors grown intradermally on their right shoulders. HPPH (0.25 mg/kg) is injected i.v. at 24 hours prior to light delivery. Initial PS concentration is quantified via interstitial PS fluorescence measurements after correction for tissue optical properties. Light is delivered by a linear source at various light doses (12-150 J/cm) with powers ranging from 12 to 150 mW per cm length. The necrosis radius is quantified using ScanScope after tumor sectioning and hematoxylin and eosin (H&E) staining. The macroscopic optimization model is used to fit the results and generate four PDT parameters. Initial results of the parameters for H460 tumors will be reported and compared with those for the RIF tumor.

8931-48, Session PMon

Chlorophyll spectra in mice fluorescence measurements for PDT

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It is critical to determine the concentration of photosensitive drug used during photodynamic therapy (PDT) for effective dose calculation. Experiments with mouse tumor models are often used to refine PDT parameters and develop techniques. During these treatments, fluorescence spectra are collected to measure the photosensitizer concentration. Fluorescence spectroscopy and PDT dosimetery can both be complicated by the presence of pheophorbide A (PhA), a product of the digestion of chlorophyll-containing food. PhA has emission peaks in the same range as many sensitizers, making it is difficult to accurately determine the intensity of the desired fluorescence peak and thus the concentration of the drug. Furthermore, PhA is another photosensitizing agent that produces singlet oxygen. For fluorescence measurements in mice, it is useful to eliminate this chlorophyll-based signal. Skin fluorescence spectra were collected to see the timeline of the PhA signal in mice on a chlorophyll-free rodent diet. In this study, the basis spectrum of PhA was characterized, and it was verified that the fluorescence signal from PhA would be eliminated after ~9 days. The intended PDT effect can be more accurately established without unintentional photochemical processes occurring from the presence of this chlorophyll-based product.

8931-49, Session PMon

Intense pulsed light an alternative to perform photodynamic therapy

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Photodynamic therapy (PDT) involves the best interaction of light, photossensitizer and singlet oxigen, produced by photochemical reactions. The photossensitizers used in PDT absorves light in different wavelenghts (Soret band and Q bands), however the irradiation procedure is done around 630 nm, wavelenght where the light penetration on tissue is greater and deeper. The successful of PDT depends on the light dose, delivered in specific wavelenght, and of the best photossensitizers. It is known that most photosensitizers presented a broad absorption band, then the aplicattion of intense pulsed light (IPL) can be usefull optimizing the PDT. There are several studies using IPL in dermatologic clinical showing best results. In this work we performed PDT using IPL in healthy models of liver (rat) and skin (pig). The porphyrin production was monitored using widefield fluorescence imaging and fluorescence spectroscopy collected at liver and skin surface, using the time of high accumulation and production of drug. The results obtained in our previous experiments showed that the PDT effectiveness using IPL indicate was satisfactory. The aplication of IPL will be usefful getting possible the clinical application of many photossensitizers that absorbs light, in different wavelenght, using the same device, as well as increasing the light absorption of PS by excitation in all bands. The investigation of PDT by histological analyses has being performed.

8931-50, Session PMon

Fluorescence lifetime for melanoma murine detection

Layla Pires, Marcelo S. Nogueira, Lilian Tan Moriyama, Cristina Kurachi D.D.S., Univ. de São Paulo (Brazil)

Fluorescence lifetime measurement is a technique that has been presented as potential tool to improve cancer diagnostics, especially for breast cancer. In this study, we evaluate the efficacy of fluorescence lifetime for melanoma diagnostics in an in vivo melanoma model. Murine melanoma cells were intradermically injected at nude mice. The spectroscopy system is based on the laser excitation at 378nm and 442nm, targeting the tissue detection of NADH and FAD molecules. Tumor development and treatment response after photodynamic therapy was monitored. After melanoma cell injection, the optical measurements were taken at 24 h, 48 h, 72 h, and 7 days. Nude mice were intravenously injected with chlorine, and illumination at 660 nm performed after 1 h. After PDT, the fluorescence lifetime measurements were taken at immediately, 24 h, and 48 h. The NADH/FAD ratio is changed during melanoma progression, high differences are observed at longer melanoma implantation. These results show the potential of the use of the fluorescence lifetime to evaluate melanoma progression and treatment response. Financial support: FAPESP - CEPID Program.

8931-51, Session PMon

Comparison of photodynamic therapy using two photosensitizers for melanoma: in vitro study

Layla Pires, Bruno Ono, Lilian Tan Moriyama, Cristina Kurachi D.D.S., Univ. de São Paulo (Brazil)

Melanoma skin cancer is responsible for 80 to 85% of the death caused by skin cancer. Treatment options are based on surgery and chemotherapy but the survival is approximately 6 months after the diagnostic. In this way, the development of new techniques for melanoma treatment is highly important. The in vitro effect of photodynamic therapy in two melanoma cell lines, a murine melanoma B16F10 and a human melanoma G361 was investigated. Three photosensitizers were tested: Photodithazine (a glucamine salt chlorine), Photogem (haematoporphyrin) and a chlorine e6. The photosensitizer kinetic was studied using confocal microscopy. Tested drug-light intervals were of 30 min, 1 h, 2h, 6h, 12h and 24 h. The irradiation was performed with a LED equipment emitting at 630nm for Photogem and at 660nm for the chlorines. Cell survival fraction was evaluated by MTT test. Chlorines were more uptake by the melanoma cell when comparing to porphyrin. Citotoxic assays showed that at low concentrations the photosensitizers did not show dark toxicity. PDT induced death of approximately 99% of the cells with a single irradiation session. These results indicate that chlorines are more effective for in vitro melanoma inactivation than porphyrin and that this sensitizer may be a good option for improving the PDT effect on melanoma. Financial support: Capes.

8931-52, Session PMon

Synthesis and study of novel targeted photosensitive for use in invasive breast cancer

Rebecca Gilson, Rui Tang, Pinaki Sarder, Samuel Achilefu, Washington Univ. School of Medicine in St. Louis (United States)

Photodynamic therapy (PDT) is a promising treatment modality that can be targeted and activatable, mitigating the destruction of healthy tissues that commonly occurs in other clinical cancer therapies. In this study, we evaluate the efficacy of using a peptide ligand to target the photosensitizer Chlorin e6 (Ce6) to cancer cells. This approach provides greater contrast between tumor and uninvolved tissue, while increasing the concentration of the photosensitizer in tumor cells. Our results show that in the Ce6-peptide conjugate maintained Ce6's photophysical properties, including pH sensitivity in absorption, emission, and fluorescence lifetime modes. In vitro, the Ce6-peptide conjugate selectively internalizes in cancer cells. In addition, we have compared the subcellular localization of Ce6 and Ce6-peptide conjugate.

8931-53, Session PMon

Evaluating the efficacy of photodynamic therapy in glioblastoma spheroids

Kohei Watanabe, Bryan Q. Spring, Massachusetts General Hospital (United States) and Wellman Ctr for Photomedicine, Harvard Medical School (United States); Srivalleesha Mallidi, Wellman Ctr for Photomedicine, Harvard Medical School (United States) and Massachusetts General Hospital (United States); Dmitriy Timerman, Harvard-MIT Health Sciences and Technology (United States); Tayyaba Hasan, Wellman Ctr for Photomedicine, Harvard Medical School (United States) and Massachusetts General Hospital (United States)

Recent studies have shown that the cancer stem cells identified in glioblastoma tissues (glioblastoma stem cells; GSC) are potent tumor initiators and also the source of the tumor recurrence. Studies have shown that GSC identified by CD133 immunostaining, a biomarkers associated with glioblastoma stem cells, exists in various glioblastoma cell lines. It has also been shown that the tissue culture condition affects the population of the GSCs and often more CD133+ GSCs are observed in spheroid culture compared to monolayer culture. Since spheroid culture has a more complex microenvironment in its three dimensional structure, treatment response is often different from that of monolayer culture and could be representative of in-vivo conditions. Photodynamic therapy (PDT) is a promising cancer therapeutic strategy that uses a photosensitizer and light. Clinical trials of glioblastoma-PDT using non-fluorescent prodrug (5-aminolevulinic acid, ALA), which is converted into fluorescent and photodynamic porphyrins (protoporphyrin IX, PpIX), have shown promising outcome. In this study, we carried out preliminary experiments with glioblastoma spheroid cultures to evaluate the efficacy of the conversion from ALA to PpIX and the treatment response of subsequent PDT. Efficacy of ALA to PpIX conversion and PDT efficacy in CD133+ cells and CD133- cells were evaluated using flow cytometry. We will further develop this approach by modulating the biology of the GSC subpopulation for enhanced ALA-to-PpIX conversion.

8931-54, Session PMon

Targeting glioblastoma stem cells for fluorescence-guided resection with follow-up photodynamic therapy: preliminary studies

Bryan Q. Spring, Wellman Ctr. for Photomedicine, Harvard Medical School (United States) and Massachusetts General Hospital (United States); Kohei Watanabe, Massachusetts General Hospital (United States) and Wellman Ctr for Photomedicine, Harvard Medical School (United States) and Canon, Inc. (United States); Srivalleesha Mallidi, Massachusetts General Hospital (United States) and Wellman Ctr. for Photomedine, Harvard Medical School (United States); Dmitriy Timerman, Massachusetts General Hospital (United States) and Wellman Ctr. for Photomedicine, Harvard Medical School (United States); Tayyaba Hasan, Massachusetts General Hospital (United States) and Wellman Ctr. for Photomedicine, Harvard Medical School (United States) and Harvard-MIT Health Sciences and Technologies (United States)

No Abstract Available

Conference 8932: Mechanisms for Low-Light Therapy IX

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8932 Mechanisms for Low-Light Therapy IX

8932-1, Session 1

Application and possible mechanisms of combining LLLT, Infrared hyperthermia, and ionizing radiation in the treatment of cancer *(Invited Paper)*

Edward H. Abraham, Van H. Woo, Cheryl Harlin-Jones, Artesian Cancer Ctr. (United States); Anja Heselich, Florian Frohns, Technische Univ. Darmstadt (Germany)

Benefit of Concomitant Infrared Hyperthermia/LLLT (Low Level Laser Therapy) And Ionizing Radiation Therapy.

Purpose/Objectives: Presentation with locally advanced bulky superficial tumors is clinically challenging. To enhance the efficacy of chemotherapy and IMRT (Intensity Modulated Radiaiton Therapy), we have developed an inexpensive and clinically effective infrared hyperthermia approach that combines black-body with halogen and discrete wavelength infrared clinical laser (LLLT) sources to produce a composite spectrum extending from far-infrared to near-infrared with discrete penetrating wavelengths generated by clinical infrared lasers 810 nm and/or 830 nm. This composite infrared spectrum from three sources is applied before and after radiation therapy. We monitor the surface and deeper temperature with thermal probes but use the surface temperature as the thermal constraint.

Materials/Methods: Infrared heat lamp (Tempco, CRE10002 nonluminous 250W IR lamp with 950 °F gray-body distribution) and halogen lamp (GE 17986 100-Watt 1500-lumen Halogen Bulb) and infrared lasers (Thor 810 nm or microlight 830 nm). Temperature probes from Deastron-Fearing Corporation and Fluke 63 Infrared Thermometry.

Results: 30+ patients with squamous and basal cell carcinomas of the head and neck including oral cavity, melanomas, breast cancer and tumors with infrared 20 minutes pre and post IMRT tumor dose up to 70 Gy at 2 Gy/fraction. We monitored the tumor response by direct caliper assessment, photographs, ultrasound and CT scan and/or PET scan measurements. We present detailed data for a patient treated with infrared and IMRT for 2 weeks, then treated with IMRT as mono-therapy, and then with resumption of the combination infrared/hyperthermia and IMRT therapy. Patient had breast cancer chest wall recurrence including 10 tumor nodules caliper assessable.

The nodules had maximal shrinkage rates with combination therapy and slower tumor shrinkage rates during radiation mono-therapy. No significant adverse reactions observed (common toxicity criteria (CTC) < 2). Tumor shrinkage (change in cross-section (cm-squared): -0.61 AVG (cm-squared/week) IMRT with infrared light/heat versus -0.18 AVG (cm-squared/week) IMRT without infrared light or heat T-TEST: p<.0065 for n=10 evaluable breast tumor nodules.

Conclusions: We have developed an infrared/laser/hyperthermia system for the administration of infrared radiation/hyperthermia before and after IMRT. We have observed that this combined approach enhances tumor shrinkage with bulky tumors extending to 4 cm and and in some casses deeper. We have developed a clinical method to confirm the enhancement of the IMRT by the infrared/hyperthermia. We are further defining the relative contributions and molecular mechanisms the effects of both modulation of intracellular and extracellular ATP (adenosine triphosphate) levels modulated by discrete components of the infrared spectrum, to interact and enhance the effectiveness of hyperthermia and the radiation therapy in tumor and normal tissue models. Some further clinical applications will be discussed.

8932-2, Session 1

Near infrared laser therapy for stroke: does it penetrate the skull?

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Transcranial near infrared laser therapy (NILT) has been studied in 3 clinical trials, NeuroThera Effectiveness and Safety Trial (NEST)-1, NEST-2 and NEST-3, as a novel neuroprotective treatment for acute ischemic stroke (AIS). However, despite NILT efficacy in animals and NEST-1-2, NEST-3 was halted due to futility. The differential effects of NILT in clinical trials suggest that the power density (Pd) used in animals may have been insufficient to promote neuroprotection and clinical improvement in a diverse population of stroke patient. We present our findings on the penetration characteristics of NILT through the skull of 4 different species (mouse, rat, rabbit and human). We used a variable Power (W) K-Laser Inc 800nm wavelength device for the studies to determine the effects of NILT using Pd's of 5-700 mW/cm2. we measured significant attenuation of NILT penetration from 38.8-39.8% to 11.0-11.8% as skull thickness increased from 0.441± 0.033 mm in mouse to 2.115 mm± 0.309 mm in rabbit. In human calvaria, where thickness ranged from 5.21-10.24mm, there was further attenuation of NILT penetration down to 3.27-3.63% of applied Power. In conclusion, this study suggests that NILT translation from non-human species to humans cannot be done in a straight forward manner. Due to a huge variability in the thickness of the human skull, NILT penetration characteristics are diverse. It is possible that NEST clinical trials were not adequately optimized to allow for sufficient NILT penetration in the range of those that were previously shown to be effective in rodent and rabbit translational stroke studies.

8932-3, Session 1

Photo-excitation of electrons as a theory of the mechanism of the increase of ATP production in mitochondria by laser therapy

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The hypothesis explains the molecular basis for restoring mitochondrial function by laser therapy. It also explains how laser therapy reverses both excessive oxidation (lack of NADH/FADH2) and excessive reduction (lack of O2) states of cytochrome c oxidase complex. It is proposed that photons interact with heme molecules of cytochrome c oxidase. A molecule of heme contains a porphyrin ring and an atom of iron in the center. The iron atom (Fe) can switch oxidation states back and forth between ferrous (Fe2+) and ferric (Fe3+) by accepting or releasing an electron. The porphyrin ring is a complex aromatic molecule that has 26 pi electrons which are "delocalized", spinning in the carbon rings creating a resonating electromagnetic cloud. Photons with similar wavelengths are absorbed by the cloud increasing its energy. The energy is then passed on to the centrally located atom of iron existing in a reduced state (Fe2+). The electrons on the orbits of the iron atom accept this electromagnetic energy, and change orbitals to a higher energetic level. If the energy is sufficient, electrons leave the atom entirely. If this occurs, Fe2+ become oxidized to Fe3+ releasing electrons, thus restoring electron flow and the production of ATP. At the same time, electrons freed from complex IV may have sufficient energy to be picked by NAD+/FADH and re-enter the chain at the complex I or II amplifying the flow of electrons.

8932-4, Session 1

Combination of nitric oxide therapy, anti-oxidative therapy, low level laser therapy, plasma rich platelet therapy and stem cell therapy as a novel therapeutic application to manage the pain and treat many clinical conditions

Salaheldin Halasa, Cutting Edge Treatment Ctr. (United States) and Compassionate Pain Management (United States); Eva Dickinson, Compassionate Pain Management (United States) and Cutting Edge Treatment Ctr. (United States)

Research Strategy: is conducting series of human clinical trials of combining nitric oxide therapy, anti-oxidative therapy, low level laser therapy, plasma rich platelet therapy and stem cell therapy to manage the pain and treat many clinical conditions.

Specific Aim 1 will establish the optimal laser, nitric oxide donor, antioxidant, PRP and stem cells parameters for maximizing the efficacy of the therapies. Specifically we will study the effect of each individual therapeutic modality and compared with the combined therapeutic modalities. Specific aim 2 will investigate the mechanisms of action of low level laser therapy, anti-oxidative therapy, nitric oxide therapy, PRP therapy and stem cell therapy

Innovation: This proposal has a high level of innovation. The synergic interaction of the combined therapies is phenomena Nitric oxide and redox mechanisms in the immune response

1. ?NO/ROS??TH2-M2/TH1 –M1??humeral immunity.

2. ?NO/ROS??c-GMP ??TH1-M1/TH2 –M2 ? ?cytotoxic-Immunity +?anticancer.

3. ?NO + ?ROS= ?RNS.

4. ?RNS/NO?? Nitrosative??COX+?LOX ? ?PGE2??c-AMP?? inflammation+ ?Tissue destruction+ ?antimicrobial+ ?anticancer +?cytotoxic-Immunity +?apoptosis.

5. ?NO/RNS? ?COX2+?COX1+?LOX??PGE2??c-AMP? ?Anti-inflammation +?Tissue restoration+ ?angiogenesis +?proliferation+ ?anti-apoptosis+ ?Telomerase activity.

Mechanism of Photobiomodulation therapy:

1- Infrared LLLT stimulate the ETC reactions ??ATP+?NADH+ ?ROS+ ?NO? stimulation of growth factor gens ??growth and proliferation.

2- Red LLLT stimulates ECT reaction by ?NO photo disassociation from the CCO ??ATP+?NADH +?NO+ ?ROS? stimulation of growth factor gens ?? growth and proliferation.

3- Blue LLLT ??NO disassociation from the Hb ??NO +?shift deoxy -Hb? oxy-Hb. ?? growth and proliferation.

4- Green LLLT?? Na/k ATPase??ATP utilization + ?membrane transportations+ improve the cell rheology and integrity?? growth and proliferation.

8932-5, Session 1

VCSELs in the visible to IR as a light source for low light therapy

Mary Hibbs-Brenner, Klein L. Johnson, Vixar Inc. (United States); Matthew M. Dummer, Vixar Inc (United States); William K. Hogan, Charles Steidl, Vixar Inc. (United States)

VCSELs provide a very versatile optical source for Low Light Therapy applications. This talk will discuss performance characteristics and packaging demonstrations for VCSELs primarily operating in the 680nm and 850nm regimes. At 680nm individual VCSELs produce >10mW, while >0.35W can be provided from a 0.4mm2 array emission area. Spectral width is typically 1-2nm even for a multi-mode or array device. At 850nm these numbers increase to >30mW and >0.8W. Even higher powers can be achieved under pulsed modulation, i.e. 0.55W for a 680nm VCSEL array or 1.2W for an 850nm VCSELarray. While we report on results achieved at 680nm and 850nm, extension to wavelengths ranging from 660nm to 1000nm is easily achieved.

The packaging flexibility of VCSELs also makes them of significant interest to the Low Light Therapy community. We will report on the incorporation of VCSELs into surface mount packages, including typical LED packages such as the PLCC, or ceramic chip carriers. VCSELs in PLCC packages have been attached to flexible circuits to provide a broad area illumination. We will also report on a unique chip on board package which easily allows for the addition of optical elements such as diffusers, diffraction gratings or lenses. This package is 2mm on a side, sufficiently small for incorporation into catheters or implantation. This flexible package platform can, for example, provide the ability to couple light into a fiber with >90% coupling efficiency with the use of a lens, or can be used to provide broad area illumination with the use of a diffuser. Extensive reliability testing under CW, pulsed and humid conditions will also be reported.

8932-6, Session 1

Near infrared laser penetration and absorption in human skin

Babak Nasouri, The Univ. of Texas at Austin (United States); Thomas Murphy, The University of Texas at Austin (United States); Halil Berberoglu, The Univ. of Texas at Austin (United States)

For understanding the mechanisms of low level laser/light therapy, accurate knowledge of light interaction with tissue is necessary. In this talk, we present an experimentally validated three dimensional, multi-layer Monte Carlo simulation tool for studying light penetration and absorption in human skin.The skin is modeled as a three-layer participating medium (epidermis, dermis, and subcutaneous) where its geometrical and optical properties are obtained from the literature. Both refraction and reflection are taken into account at the boundaries according to Snell's law and Fresnel relations. A forward Monte Carlo method was implemented and validated for accurately simulating light penetration and absorption in absorbing and anisotropically scattering media. Local profiles of light penetration and volumetric absorption densities were simulated for uniform as well as Gaussian profile beams with different spreads at 155 mW average power over the spectral range from 1000 nm to 1900 nm. The results show the effects of beam profiles and wavelength on the local fluence within each skin layer. Particularly, the results identify different wavelength bands for targeted deposition of power in different skin layers. Finally, we show that light penetration scales well with the transport optical thickness of skin. We expect that this tool along with the results presented will aid researchers resolve issues related to dose and targeted delivery of energy in tissues.

8932-7, Session 1

Evaluation of laser photobiomodulation (? 780 nm) in the repair of dental reimplantation in rats

Fabíola B. Bastos de Carvalho, Rebeca M. Vasconcelos, Laila S. Santos, Artur F. S. Barbosa, Marcio C. Aguiar, Maria Cristina T. Cangussu, Antônio L. B. Pinheiro, Luciana M. Pedreira Ramalho, Univ. Federal da Bahia (Brazil)

The success of tooth reimplantation is limited, most of the teeth is lost due to progressive external root resorption. The aim of this study was

to assess histologically the effect of laser photobiomodulation on repair after tooth reimplantation. 60 Wistar Albinus rats had the right upper incisor extracted and then divided into 4 groups: G1 - absence of storage medium; G2 - milk as storage medium; G3 - milk as storage medium followed by a GaAlAs laser irradiation on dental surfaces and at the entrance of alveolus (? = 780 nm; P = 70 mW; CW; DE = 21,7 J/cm2); G4 - milk as storage medium, laser irradiation like G3 before reimplantation and after this procedure on the buccal and palatal mucosa (8,4 J/cm2 per session) every 48 hours for 15 days. The animals were killed 15, 30 and 60 days after reimplantation. The results showed that after 15 days G4 exhibit a more intense chronic inflammation, with the presence of clastic cells and moderate inflammatory root resorption ($p<0.05$) when compared to G3, which has been observed absence of these parameters. At 30 days in G1, G2 and G4 was observed chronic inflammation of mild to moderate and severe external root resorption. G3 remained with no inflammation and inflammatory root resorption at 30 and 60 days. It is concluded that laser irradiation on the dental surface and the entrance of the alveolus prior to reimplantation has a positive biomodulative effect on the healing process after tooth replantation in rats.

8932-8, Session 1

Wavelength, beam size and type dependences of cerebral low-level light therapy: A Monte Carlo study on visible Chinese human

Ting Li, Yue Zhao, Meixue Duan, Yunlong Sun, Kai Li, Univ. of Electronic Science and Technology of China (China)

Low level light therapy (LLLT) has been clinically utilized for many indications in medicine requiring protection from cell/tissue death, stimulation of healing and repair of injuries, pain reduction, swelling and inflammation. Presently, use of LLLT to treat stroke, traumatic brain injury, and cognitive dysfunction is attracting growing interest. Near-infrared light can penetrate into the brain tissue, allowing noninvasive treatment to be carried out with few treatment-related adverse events. Optimization of LLLT treatment effect is one key issue of the field; however, only a few experimental tests on mice for wavelength selection have been reported. We addressed this issue by low-cost, straightforward and quantitative comparisons on light dosage distribution in Visible Chinese human head with Monte Carlo modeling of light propagation. Optimized selection in wavelength, beam type and size were given based on comparisons among frequently-used setups (i.e., wavelengths: 660 nm, 810 nm, 980 nm; beam type: Gaussian and flat beam; beam diameter: 2 cm, 4 cm, 6cm).This study provided an efficient way to guide optimization of LLLT setup and selection on wavelength, beam type and size for clinical brain LLLT.

8932-9, Session 2

Irradiation at 660 nm modulates different genes central to wound healing in wounded and diabetic wounded cell models

Nicolette N. Houreld, Univ. of Johannesburg (South Africa)

Wound healing is a highly orchestrated process and involves a wide variety of cellular components, chemokines and growth factors. Low-intensity laser irradiation (LILI) has influenced gene expression and release of various growth factors, cytokines and extracellular matrix proteins involved in wound healing. This study aimed to determine the expression profile of genes involved in wound healing in wounded and diabetic wounded fibroblast cells in response to irradiation at a wavelength of 660 nm. Human skin fibroblast cells (WS1) were irradiated with a diode laser (wavelength 660 nm; fluence 5 J/cm2; power output 100 mW; power density 11 mW/cm2; spot size 9.1 cm2; exposure duration 7 min 35 s). Total RNA was isolated and 1 µg reverse transcribed into cDNA which was used as a template in real-time qualitative polymerase chain reaction (qPCR). Eighty four genes involved in wound healing (extracellular matrix & cell adhesion; inflammatory cytokines & chemokines; growth factors; and signal transduction) were evaluated in wounded and diabetic wounded cell models. Forty eight hours post-irradiation, 4 genes were significantly up-regulated and 8 genes were down-regulated in irradiated wounded cells, whereas 1 gene was up-regulated and 33 genes down-regulated in irradiated diabetic wounded cells. Irradiation of stressed fibroblast cells to a wavelength of 660 nm and a fluence of 5 J/cm2 modulated the expression of different genes involved in wound healing in different cell models. Modulation of these genes leads to the effects of LILI seen both in vivo and in vitro, and facilitates the wound healing process.

8932-10, Session 2

Genetic expression of adipose derived stem cell and smooth muscle cell markers to monitor differentiation potential following low intensity laser irradiation

Heidi Abrahamse, Univ. of Johannesburg (South Africa)

Mesenchymal stem cells have the capacity to differentiate into a variety of cell types that could potentially be used in tissue engineering and regenerative medicine. Low intensity laser irradiation (LILI) has been shown to induce a significant increase in cell viability and proliferation. Growth factors such as retinoic acid (RA) and transforming growth factor ?1 (TGF?1) play important roles in the differentiation of cells. The aim of this study was to investigate whether LILI in combination with growth factors could induce the differentiation of adipose derived stem cells (ADSCs) co-cultured with smooth muscle cells (SMCs). The study used primary and continuous ADSC cell lines and a SMC line (SKUT-1) as control. Cells were co-cultured directly at a ratio of 1:1 using established methods, with and without growth factors and then exposed to LILI at 5 J/cm2 using a 636 nm diode laser. The cellular morphology, viability and proliferation of the co-cultures were assessed over a period of one week. The study also monitored the expression of cell specific markers over the same period of time. Genetic expression of the markers for both adipose derived stem cells (?1 Integrin) and smooth muscle cells (Smoothelin, Desmin, Heavy Myosin Chain and Smooth Muscle Alpha Actin) was monitored using RT-PCR. Cell viability and proliferation increased significantly in the co-cultured groups that were exposed to laser alone, as well as in combination with growth factors. Furthermore, there was a significant decrease in the expression of stem cell markers in the ADSCs over time. The results indicate that LILI in combination with growth factors not only increases the viability and proliferation of co-cultured cells but also decreases the expression of ADSC stem cell markers. This could indicate the possible differentiation of ADSCs into SMCs.

8932-11, Session 2

Alterations in cell migration and cell viability of wounded human skin fibroblasts following visible red light exposure

Vijendra Prabhu, Satish Bola Sadashiva Rao, Krishna Kishore Mahato, Manipal Univ. (India)

The present study intended to examine the effect of visible red light on structural and cellular parameter on wounded skin fibroblast cells. To achieve the stated objective, uniform scratch was created on confluent monolayered human skin fibroblast cells, and were exposed to single dose of He-Ne laser (15 mm spot, 6.6808 mWcm-2) at 1, 2, 3, 4, 5, 6 and 7 Jcm-2 in the presence and absence of 10 % fetal bovine serum (FBS). Beam profile measurements of the expanded laser beam were conducted to ensure the beam uniformity. The influence of laser dose on the change in temperature was recorded using sensitive temperature probe.

Additionally, following laser exposure cell migration and cell survival were documented at different time intervals on wounded human skin fibroblast cells grown in vitro. Beam profile measurements indicated more or less uniform power distribution over the whole beam area. Temperature monitoring of un-illuminated control and laser treatment groups displayed negligible temperature change indicating the absence of thermal effect at the tested laser doses. In the absence of 10% FBS, single exposure of different laser doses failed to produce any significant effects on cell migration or cell survival. However, in the presence of serum single exposure of 5 J/cm2 on wounded skin fibroblasts significantly enhanced the cell migration (P<0.05) compared to the other tested doses (1, 2, 3, 4, 6 & 7 J/cm2) and un-illuminated controls. In conclusion, the LLLT acts by improving cell migration and cell proliferation to produce measurable changes in wounded fibroblast cells.

8932-12, Session 3

Nitric oxide measurements in hTERT-RPE cells and subcellular fractions exposed to low levels of red light

Jeffrey C. Wigle, U.S. Air Force (United States); Cherry C. Castellanos, Michael L. Denton, TASC, Inc. (United States); Eric A. Holwitt, U.S. Air Force Academy (United States)

Exposure of hTERT-RPE1 cells to 2.88 J/cm2 of 637-nm light, resulted in an induced resistance to lethal photothermal challenge at 2.0 um. Changes in expression of various genes associated with apoptosis have been observed, but the biochemical link between light absorption and gene expression is unknown. It is postulated that absorption of the red light by cytochome c oxidase (CCOX) facilitates displacement of nitric oxide (NO) by O2 in the active site of CCOX, increasing cellular respiration and intracellular ATP. NO is a known second messenger that cells can synthesize from l-arginine using NO synthases, so we measured NO levels in whole cells and subcellular fractions with and without exposure to red light. A Qiagen mitochondria isolation kit was used to fractionate cells and DAF-FM, a fluorescent dye that stoichiometrically reacts with NO, was used to measure NO levels in whole cells and subcellular fractions: cytosolic proteins, nuclei, mitochondria, microsomes and mitochondria wash. Red light induced a small, but consistently reproducible, increase in fluorescence intensity in whole cells and all subcellular fractions. Whole cells exhibited the highest overall fluorescence intensity followed by cytosolic proteins, microsomes, nuclei and mitochondria (which were about equal), then mitochondria wash. At this time it is undetermined if red light is stimulating NO synthases, cellular esterases (which activate the DAF-FM before it reacts with NO) or both. NO synthase inhibitors will be used to determine which enzymes are being affected by the red light.

8932-13, Session 3

In vitro effect of 470 nm LED (Light Emitting Diode) in keloid fibroblasts

Fabianne Furtado, Silvilena Bonatti, Bernardo S. Hochman, Lydia M. Ferreira, Univ. Federal de São Paulo (Brazil)

Purpose: To quantify keloid fibroblasts after irradiation with 470nm blue LED, in vitro.

Methods: Fibroblasts from keloid and adjacent skin have been obtained from 6 patients. Cells have been cultivated and maintained in DMEM culture medium. In Petri dishes, they were irradiated with energy doses of 6J, 12J and 18J. After 24 h, counting was done by the average of the triplicates for each sample.

Results: There were no differences in the number of fibroblasts between the groups, regardless of the energy levels used (p<0,626). Conclusion: 470nm blue LED did not change the number of keloid fibroblasts, irradiated at doses of 6J, 12J and 18J, 24 h after irradiation.

8932-14, Session 3

Programmed cell death mechanism identified in breast, lung and colon cancer cells post photodynamic therapy using PCR arrays

Heidi Abrahamse, Univ. of Johannesburg (South Africa)

Photodynamic therapy (PDT) is a noninvasive form of cancer therapy, successfully applied for the treatment of various cancer types. Zinc phthalocyanine (ZnPcSmix) was used as the photosensitizer (PS) in this study to investigate the cell death patterns as a result of PDT in breast, lung and colon cancer cell lines (MCF-7; A549; DLD-1) in vitro using a 680 nm diode laser at a fluence of 5 J/cm2. Flow cytometry using Annexin V- fluorescein isothiocyanate (FITC), a cell death immunosorbent assay (ELISA) and gene expression analysis following ZnPcSmix mediated PDT were performed to determine the induced cell death pathways. Cells treated with light activated ZnPcSmix resulted in a significant production of ROS and a dose dependant decrease in viability and proliferation as well as an increase in cell membrane damage. Subcellular localization studies indicated the lysosomes and mitochondria as the main targets for PS localization. The apoptotic cells abounded after the treatment, nuclear fragmentation were seen as oligonucleosomal degradation and increased expression of the Bcl-2, DFFA1 and CASP-2 genes, indicated that apoptosis is the main induced mode of cell death. Moreover, there was a significant increase in both cathepsin D and cytochrome C at 1 and 24 h. ZnPcSmix mediated PDT led to an apoptotic cell death pathway and the PS used showed its ability to stimulate and initiate programmed cell death.

8932-15, Session 3

Association phenothiazine and laser on growth of C. tropicalis fluconazole-resistant

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Candidiasis is caused by Candida species found on the skin, gastrointestinal tract and mucous cavities of the humans and may be acute, chronic, localized or systemic. Alhough C. albicans is the species most often identified as responsible for this type of infection C. Tropicalis has been considered an emerging cause. The effect of the association of phenothiazine - PTZ and laser on fluconazole-resistant C.tropicalis growth was tested. 2.5 x 106 CFU / mL 100 mg / mL of phenothiazine with the pre-irradiation time of 10 min were irradiated with laser light (? 660 nm; 4.8 and 12 J/cm2 (L1 and L2 respectively) 40 mW) followed by incubation in RPMI for 24h. The following conditions were tested: control (LF-), laser (L + F-), phenothiazine (L-F +), and PACT (F + L +). Statistically significant diferences were seen between groups (L-F +) and (F + L +) for both conditions of the laser, with a growth inhibition of the yeast around 67 and 51%, respectively, however, when using only the laser there was an increase of 18% in the survival of these cells. PACT´s efficacy on fluconazole-resistant C. tropicalis depended on both the time of pre-irradiation and concentration of the PTZ.

8932-16, Session 3

In vitro influence of photodynamic antimicrobial chemotherapy on staphylococcus aureus by using phenothiazines derivatives associated with laser/LED Light

Susana P. de Oliveira, Juliana S. Monteiro, Gustavo M. Pires Santos, Univ. Federal da Bahia (Brazil); Fernando J. P. Sampaio D.D.S., Univ. Federal da Bahia (Brazil) and National Institute of Optics and Photonics (Brazil); Rafael Araújo Gomes Júnior, Fundação Oswaldo Cruz (Brazil); Maria F. M. Gesteira D.D.S., Univ. Federal da Bahia (Brazil) and National Institute of Optics and Photonics (Brazil); Aldo Brugnera Jr., Univ. do Vale do Paraíba (Brazil); Fátima A. A. Zanin, Univ. Federal da Bahia (Brazil) and National Institute of Optics and Photonics (Brazil) and Brugnera and Zanin Institute (Brazil); Marcos André V. Vannier-Santos, Fundação Oswaldo Cruz (Brazil); Antônio Luiz B. Pinheiro, Univ. Federal da Bahia (Brazil)

The objective of this study was to evaluate the bactericidal effect of photodynamic antimicrobial chemotherapy (PACT) using phenothiazinium dyes - PTZ irradiated with red laser (?660nm) or red-orange LED (?632±2nm) on Staphylococcus aureus in vitro. triplicate tests were performed in 10 groups: control, Laser (L1+P- and L2+P-) bacterial suspensions were irradiated only with laser energy 2.4 and 4.8 J/cm2 respectively, (Led1+P- and Led2+P-) irradiated only with LED energy 2.4 and 4.8 J/cm2 respectively, (L1+P+ and L2+P+) irradiated with laser in the presence of 1?g/ml of photosensitizer, (Led1+P+ and Led2+P+) irradiated with LED in the presence of 1?g/ml of photosensitizer and finally (L-P+) only in the presence of PTZ dye. Bactericidal effect of the PACT was assessed by counting colony-forming units. The results showed no significant difference on regards different energy densities on group PACT for both lights. PACT groups (L2+P+ and Led2+P+) compared to the Control showed significant reduction of CFUs. LED/Laser groups (L2+P- and Led2+P-) compared to control and PTZ groups showed also significant differences as groups LED/Laser (4.8J/cm2) increased the average of CFUs. Although the results of this study showed reduction of CFUs when using appropriate Laser or LED-dye treatment combination, it needs further investigation.

8932-17, Session 4

Can light interact with neurons to decrease pain? *(Invited Paper)*

Marcelo V. Pires de Sousa, Univ. de São Paulo (Brazil) and Wellman Ctr. for Photomedicine (United States); Cleber Ferraresi, Beatriz Kaippert, Masayoshi Kawakubo, Wellman Ctr. for Photomedicine (United States); Elisabeth M. Yoshimura, Univ. de São Paulo (Brazil); Michael R. Hamblin, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States)

The incidence of chronic pain is rising in developed countries, while acute and inflammatory pain is a common concern everywhere. Therefore control of pain (nociception) is a major medical and economic challenge for clinicians and researchers. Several studies have demonstrated the effectiveness of low-level laser (light) therapy (LLLT) to decrease many types of pain in different body locations, but the mechanisms for the pain relieving effect are still not clear. We carried a search for these mechanisms using a mouse model of measuring pain threshold employing von Frey filaments. Doing so, we have found new ways of treating, like transcranial LLLT and transcutaneous dorsal root ganglion (DRG) LLLT for pain relief, and have found strong evidence for the direct interaction of light with neurons as the major cause of pain attenuation. Performing LLLT on DRG we observed increase of pain threshold that goes up to five times and remains up to six hours. Increase in pain threshold was obtained with irradiation in several locations on the pathway of the neurons going from the evaluated paw to the brain. The evidences of cellular and molecular effects of LLLT are sustained by Immunohistochemistry assays of tubulin, Prostatic acid phosphatase, glutamate and beta endorphin.

8932-18, Session 4

Treating metabolic syndrome's metaflammation with low level light therapy: preliminary results

Tania M. Yoshimura, Univ. de São Paulo (Brazil); Alessandro M. Deana, UNINOVE (Brazil); Martha S. Ribeiro, Instituto de Pesquisas Energéticas e Nucleares (Brazil)

Metabolic syndrome comprises a constellation of morbidities such as insulin resistance, hyperinsulinemia, atherogenic dyslipidemia, dysglycemia and obesity (especially abdominal). Metabolic alterations are observed in major insulin target organs, increasing the risk of cardiovascular diseases, type-2 diabetes and therefore mortality. Tissue alterations are characterized by immune cells infiltrates (especially classically activated macrophages). Released inflammatory mediators such as TNF-? and IL-1? induce chronic inflammation in subjects with metabolic syndrome, since inflammatory pathways are activated in the neighboring cells. The intra-abdominal adipose tissue appears to be of particular importance in the onset of the inflammatory state, and strategies contributing to modulate the inflammatory process within this adipose tissue can mitigate the metabolic syndrome consequences. That being the scope of this study, male C57BL/6 and BALB/c mice were submitted to a high-fat/high-fructose diet among 8 weeks to induce metabolic syndrome. Animals were then irradiated on the abdominal region during 21 days using a 850 nm LED (300 seconds per session, 60 mW output power, ~6 J/cm? fluence, ~19 mW/cm? fluence rate). Before and during treatment, blood was sampled from the retro-orbital plexus for glucose, total cholesterol and triglycerides analysis. So far our results indicate no alterations on these metabolic parameters after low level light therapy. For further investigations, blood was collected for plasma TNF-? and IL-1? quantification, and fresh ex vivo samples of liver and intra-abdominal adipose tissue were harvested for immunohistochemistry purposes.

8932-19, Session 4

Light emitting diode ? 850nm on repair of calcaneus tendon in rats

Carlos E. Pinfildi, Rafael C. Gobbato, Michele A. Nishioka, Bernardo S. Hochman, Univ. Federal de São Paulo (Brazil)

Background: Calcaneous tendon rupture is thought like a severe lesion due the poor blood supply that can lead weeks or months to complete rehabilitation. LED (Light Emitting Diode) have been an alternative treatment towards laser because the healing results. Objective: To assess the effect of LED (? 850nm) on calcenous tendon healing in rats. Methods: Were used 30 male rats Wistar with partial lesion on the calcanenous tendon performed with direct trauma. The animals were randomly divided in 3 groups: Group 1 Sham (LED); Group 2 (led 850nm) with energy density 10J/cm2 and Group 3 (led 850nm) with energy density 20J/cm2. The animals were treated per 6 consecutive days and on the 7th day after post lesion the tendons were removed and evaluated about collagen fibers realignment (birefringence) and collagen amount of type I and III (picrosirius). Results: Group 2 (10J/cm2) and Group 3 (20J/cm2) showed better results about fibers collagen realignment when

compared sham group (p<0.001). Groups 2 and 3 showed great amount of collagen type I than the sham group (p<0.001). There wasn´t difference when compared the treatments group between them. About collagen type III, both treatment groups (2 and 3) showed lesser amount than the sham group with p<0.01. Conclusion: LED therapy was efficient on the healing process of the partial lesion calcaneous tendon in rats, showing better fibers collagen realignment and increase of the collagen type I.

8932-20, Session 4

Raman and histological study of the repair of surgical bone defects grafted with biphasic synthetic micro-granular HA + ? - calcium triphosphate and irradiated or not with ?780 nm laser

Antônio Luiz B. Pinheiro, Luiz Guilherme P. Soares, Aparecida Maria C. Marques, Jouber Mateus S. Aciole, Jean Nunes Dos Santos, Univ. Federal da Bahia (Brazil); Landulfo Silveira Jr., Camilo Castelo Branco Univ. (Brazil)

The treatment of bone loss due to different etiologic factors is difficult and many techniques aim to improve repair, including a wide range of biomaterials and, recently, phototherapies. This work assessed, by Raman spectroscopy and histology, the mineralization of bone defects. Forty rats divided into 4 groups each subdivided into 2 subgroups according to the time of sacrifice were used. Bone defects were made on the femur of each animal with a trephine drill. On animals of group Clot the defect was filled only by blood clot, on group Laser the defect filled with the clot was further irradiated. On animals of groups Biomaterial and Laser + Biomaterial the defect was filled by biomaterial and the last one was further irradiated (?780 nm, 70 mW, ? ~ 0.4 cm2, 20 J/cm2-session, 140 J/cm2-treatment). At both 15th and 30th days following sacrifice, samples were taken and analyzed by Raman spectroscopy and light microscopy. Raman peaks of inorganic and organic contents and a more advanced stage of repair were seen on group Laser + Biomaterial. It is concluded that the use of Laser phototherapy associated to biomaterial was effective to improve the repair of bone defects.

8932-21, Session 4

Evaluation of low level laser therapy irradiation parameters on rat muscle inflammation through systemic blood cytokines

Matias E. Mantineo, João P. Pinheiro, Antonio M. Morgado, Univ. de Coimbra (Portugal)

Low level laser therapy (LLLT) has been used for inflammation treatment. Here, we evaluate the effect of different doses, using continuous (830 and 980 nm) and pulsed illumination (830 nm), in the treatment of inflammation induced in the gastrocnemius muscle of Wistar rats, through cytokines concentration in systemic blood and histological analysis of muscle tissue. Animals were randomly divided into five groups per wavelength (5 animals per group: 10, 20, 30, 40 and 50 mW) plus a control group. LLLT was applied during five days, with constant exposure time and irradiated area (3 minutes; 0.5026 cm2). Blood was collected on days 0, 3 and 6. TNF-α, IL-1β, IL-2 and IL-6 cytokines were quantified by ELISA. Rats were killed on day 6. Muscle inflammatory cells were counted using optical microscopy. Treatment effects occurred for all applied doses (largest effect at 40 mW: 7.2 J, 14 J/cm2), with reduction of pro-inflammatory TNF-α and IL-1β cytokines and lower number of inflammatory cells. Results were better for 830 nm. Identical methodology was used with pulsed illumination. Average power (40 mW) and duty cycle were kept constant (80%) at five frequencies (5, 25, 50, 100 and 200 Hz). Treatment effects were observed at higher frequencies, with no significant differences between them. However, the treatment effect was lower than for continuous illumination. LLLT effect on inflammation treatment can be monitored by measuring systemic blood cytokines. A larger treatment effect was observed with continuous illumination, where results seem to be compatible with a biphasic dose response.

8932-22, Session 4

Effect of application site of low-level laser therapy in random cutaneous flap viability in rats

Bernardo S. Hochman, Rodrigo P. Prado, Carlos E. Pinfildi, Lydia M. Ferreira, Univ. Federal de São Paulo (Brazil)

This study aimed to investigate the effect of diode laser (830 nm) irradiation on the viability of ischemic random skin flaps in rats, as well as to determine the most effective site for applying laser radiation to speed healing. Methods: Seventy Wistar rats were distributed to seven groups: group 1, sham group; group 2, which received irradiation at 1 point 5 cm from the flap's cranial base; group 3, which received irradiation at 2 points (5 and 7.5 cm from the flap's base); group 4, which received irradiation at 3 points (2.5, 5 and 7.5 cm from the flap's base); group 5, which received irradiation at 1 point 2.5 cm from the flap's base; group 6, which received irradiation at 2 points (2.5 and 5 cm from the flap's base); and group 7, which received irradiation at 1 point 7.5 cm from the flap's base. The animals were subjected to laser therapy at an energy density of 36 J/cm(2) for 72 sec for five days. The percentage of necrotic skin flap area was calculated on the seventh postoperative day using a paper template. Results: The results showed that the rats in group 5 had the highest increase in skin flap viability. Statistically significant differences were not seen between any of the other groups. Conclusion: The diode laser was effective in increasing skin flap viability in rats, and laser irradiation of a point 2.5 cm from the cranial base flap was found to be the most effective.

8932-23, Session 5

Effect of low-level laser therapy with output power of 30 mW and 60 mW in the viability of a random skin flap

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Objective: To assess the effects of low-level laser therapy (LLLT) with output power of 30 and 60 mW in the viability of a random skin flap in rats. Background Data: Output power values in LLLT are not well defined. Materials and methods: Controlled, single-blind experimental study. Thirty-six animals were randomly distributed into three groups: sham group (SG), 30-mW output power (30G), and 60-mW output power (60G). In both treated groups, a fluency of 3 J/cm(2) was used. Two minutes after elevation of a random-pattern cranially based dorsal flap (4 x 10 cm), laser irradiation was applied and repeated on the first, second, third, and fourth postoperative days. Percentages of flap necrosis were calculated on the seventh postoperative day. Also, four fragments of each flap were collected to allow determination of the percentages of vascular density according to the bidimensional method of the morphometric analysis of blood vessels. Statistical analysis included the Wilcoxon test and Kruskal-Wallis variance analysis. A significance level of 5% was elected ($p < 0.05$).Results: Laser-treated animals presented significantly less necrosis than the sham group (SG, 53%; 30G, 24%; $p < 0.05$) (60G, 25%; $p < 0.05$). Also, laser-treated animals presented significantly more vascular density than the sham group (SG, 37%; 30G, 57%; $p < 0.05$) (60G, 59%; $p < 0.05$).Conclusion: LLLT (660 nm) with 30-mW and 60-mW output power was efficient in the increase of skin flap viability, but there was no difference between them.

8932-24, Session 5

LED (660 nm) and laser (670 nm) use on skin flap viability: angiogenesis and mast cells on transition line

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Skin flap procedures are commonly used in plastic surgery. Failures can follow, leading to the necrosis of the flap. Therefore, many studies use LLLT to improve flap viability. Currently, the LED has been introduced as an alternative to LLLT. The objective of this study was to evaluate the effect of LLLT and LED on the viability of random skin flaps in rats. Forty-eight rats were divided into four groups, and a random skin flap (10???4 cm) was performed in all animals. Group 1 was the sham group; group 2 was submitted to LLLT 660 nm, 0.14 J; group 3 with LED 630 nm, 2.49 J, and group 4 with LLLT 660 nm, with 2.49 J. Irradiation was applied after surgery and repeated on the four subsequent days. On the 7th postoperative day, the percentage of flap necrosis was calculated and skin samples were collected from the viable area and from the transition line of the flap to evaluate blood vessels and mast cells. The percentage of necrosis was significantly lower in groups 3 and 4 compared to groups 1 and 2. Concerning blood vessels and mast cell numbers, only the animals in group 3 showed significant increase compared to group 1 in the skin sample of the transition line. LED and LLLT with the same total energies were effective in increasing viability of random skin flaps. LED was more effective in increasing the number of mast cells and blood vessels in the transition line of random skin flaps.

8932-25, Session 5

Collagen changes and realignment induced by low-level laser therapy and low-intensity ultrasound in the calcaneal tendon

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Objective: Purpose of this study was to investigate which treatment (the exclusive or combined use of US and LLLT) most effectively contribute to tendon healing. Materials and methods: This was a controlled laboratory study with 50 rats whose Achilles tendon was injured by direct trauma. The rats were randomly divided into five groups and treated for 5 consecutive days, as follows: group 1 (control) received no treatment; group 2 was treated with US alone; group 3 was treated with LLLT alone; group 4 was treated first with US followed by LLLT; and group 5 was treated first with LLLT followed by US. On the sixth post-injury day, the tendons were removed and examined by polarized light microscopy. The organization of collagen fibers was assessed by birefringence measurements. Picrosirius-stained sections were examined for the presence of types I and III collagen. Results: There was a significantly higher organization of collagen fibers in group 2 (US) than in the control group (P = 0.03). The amount of type I collagen found in groups 2 (US), 3 (LLLT), and 5 (LLLT + US) was significantly higher than that in the control group (P < or= 0.01), but no significant differences were found between treatment groups. There were no differences in the amount of type III collagen between groups. Conclusion: Ultrasound, LLLT, and the combined use of LLLT and US resulted in greater synthesis of type I collagen; US was also effective in increasing collagen organization in the early stages of the healing process.

8932-26, Session 5

Effect of low-level laser therapy on mast cells in viability of the transverse rectus abdominis musculocutaneous flap

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To assess the effect of low-level laser therapy (LLLT) on viability of mast cells of the transverse rectus abdominis musculocutaneous (TRAM) flap. METHODS: Eighty-four Wistar rats were randomly divided into seven groups of 12 rats in each: group 1 (sham laser therapy); group 2 received 3 J/cm(2) at one point; group 3 received 3 J/cm(2) at 24 points; group 4 received 72 J/cm(2) at 1 point; group 5 received 6 J/cm(2) at 1 point; group 6 received 6 J/cm(2) at 24 points; and group 7 received 144 J/cm(2) at 1 point. All experimental groups underwent LLLT immediately after TRAM surgery and on the next two following days, for three sessions in total. The percentage of the area of skin flap necrosis was calculated on the fourth postoperative day and two samples of skin were collected from each rat with a 1-cm(2) punch to perform mast cell evaluations with toluidine blue dye. Results: Statistically significant differences were found in the percentage of necrosis, and higher values were seen in group 1 than in all other groups. Among groups 3-7 no statistically significant differences were found (p < 0.292). For mast cells, when group 1 was compared to groups 5 (6 J/cm(2) at 1 point) and 7 (144 J/cm(2) at 1 point), it had fewer mast cells. Conclusion: LLLT at a wavelength of 670 nm was effective at reducing the necrotic area, and we found that it can stimulate mast cells growth to increase vascular perfusion.

8932-27, Session 5

Low level laser therapy and light emitting diode in neuropeptides SP and CGRP secretion in healthy skin in rats

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The phototherapy effects in the skin are related to biomodulation, usually to accelerate wound healing. However, there is no direct proof of the interrelation between the effect of Low-Level Laser Therapy (LLLT) and Light Emitting Diode (LED) in neuropeptide secretion, these substances being prematurely involved in the neurogenic inflammation phase of wound healing. This study therefore focused on investigating LLLT and LED in Calcitonin Gene-Related Peptide (CGRP) and Substance P (SP) secretion in healthy skin in rats. Methods: Forty rats were randomly distributed into 5 groups: Control Group (GC), Blue LED Group (LED A), Red LED Group (LED V), Red Laser Group (Laser V) and Infrared Laser Group (Laser IV). The skin of the animals in the experimental groups was irradiated using the punctual contact technique, with a total energy of 40 J, single dose, standardized at one point in the dorsal region. After 14 minutes of irradiation, the skin samples were collected for CGRP and SP quantification using Western Blot analysis. Results: SP was released in Laser IV group (p = 0.01); there was no difference in CGRP secretion among the groups. Conclusion: Infrared (808 nm) LLLT enhances neuropeptide SP secretion in healthy skin in rats.

8932-28, Session 6

Efficacy of multiple exposure with low level He-Ne laser dose on acute wound healing: a pre-clinical study

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Investigations on the use of Low Level Laser Therapy (LLLT) for wound healing especially with the red laser light have demonstrated its pro-healing potential on a variety of pre-clinical & surgical wounds. However, until now, in LLLT the effect of multiple exposure of low dose laser irradiation on acute wound healing on well-designed pre-clinical model is not much explored. The present study aimed to investigate the effect of multiple exposure of low dose Helium Neon laser on healing progression of full thickness excision wounds in Swiss albino mice. Further, the efficacy of the multiple exposure of low dose laser irradiation was compared with the single exposure of optimum dose. Full thickness excision wounds (circular) of 15 mm diameter were created, and subsequently illuminated with the multiple exposures (1, 2, 3, 4 and 5 exposure/ week until healing) of He-Ne (632.8 nm, 4.02 mWcm-2) laser at 0.5 Jcm-2 along with single exposure of optimum laser dose (2 J/ cm-2) and un-illuminated controls. Classical biophysical parameters such as contraction kinetics, area under the curve and the mean healing time were documented as the assessment parameters to examine the efficacy of multiple exposures with low level laser dose. Experimental findings substantiated that either single or multiple exposures of 0.5 J/cm2 failed to produce any detectable alterations on wound contraction, area under the curve and mean healing time compared to single exposure of optimum dose (2 Jcm-2) and un-illuminated controls. Single exposure of optimum, laser dose was found to be ideal for acute wound healing.

8932-29, Session 6

Helium-neon laser in viability of random skin flap in rats

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Objective: The purpose of this study was to determine the role of helium-neon (He-Ne) laser random skin flap viability in rats. Materials and Methods: Experimentally controlled randomized study. Forty-eight Wistar-EPM rats were used, weighed, and divided into 4 groups with 12 rats each. The random skin flap was performed measuring 10 x 4 cm, with a plastic sheet interposed between the flap and the donor site. The Group 1 (control) underwent sham irradiation with He-Ne laser. The Group 2 was submitted to laser irradiation, using the punctual contact technique on the skin flap surface. The Group 3 was submitted to laser irradiation surrounding the skin flap, and the Group 4 was submitted to laser irradiation both on the skin flap surface and around it. The experimental groups were submitted to He-Ne laser irradiation with 3 J/cm(2) energy density immediately after the surgery and for the four subsequent days. The percentage of necrotic area of the four groups was calculated at the 7th post-operative day, through a paper-template method. Results: Group 1 reached an average necrotic area of 48.86%; Group 2, 38.67%; Group 3, 35.34%; and Group 4, 22.61%. After the statistic analysis, results showed that all experimental groups reached statistically significant values when compared to the control group, and Group 4 was the best one, when compared to all groups of this study (P<0.001).Conclusion: The He-Ne laser irradiation was efficient to increase random skin flap viability in rats.

8932-30, Session 6

Low-level laser therapy (808 nm) in incisional wound healing in rat skin

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Low-level laser therapy (LLLT) is a biophysical technique widely used for modulation of the healing process especially at red and infrared wavelengths. In vitro and in vivo LLLT studies demonstrate that low energy doses stimulate the healing process by promoting increased collagen synthesis, angiogenesis and fibroblast proliferation. In vitro studies have shown that high energy doses increased fibroblast apoptosis and reduced their proliferation and metabolism. There are few in vivo studies which elucidate the effects of high energy doses in the healing process inhibition, especially in incisional wounds, which could be an alternative in the treatment of fibroproliferative scarring. Objective: Investigate the effects of high doses of low-level laser therapy (808 nm) in incisional wound healing in rat skin. Methods: Sixty Wistar-EPM (Rattus novergecus) male rats aged 8 weeks were randomly distributed into 3 groups: (GS: simulated; GEA: 1.5J; GEB: 60J) according to the energy doses. Each group was further subdivided into two subgroups, depending on the day of sample collection (7 days: GS7, GEA7, GEB7: 14 days: GS14, GEA14, GEB14). An incision was made on the dorsum of the rats, which was irradiated for 5 consecutive days with infrared laser (808 nm), power of 100mW and cross-sectional area of the beam of 0.028 cm2. The collection of sample for analysis was from the tissue between the sutures including adjacent skin and scar tissue. Samples were sent for histological preparation for hematoxylin-eosin stain and birefringence of collagen fibers. Results: The optical path difference (OPD) was significantly higher in groups GEA and GEB regardless of the day of collection compared to group GS ($p < 0.001$). There was a decrease in the number of fibroblasts ($p < 0.001$), vases ($p < 0.021$) and in the amount of granulation tissue ($p < 0.010$) in the groups with samples collected at the 14th day in relation to groups with samples collected at the 7th day. The intensity of the inflammatory infiltrate did not change in relation to any of the doses studied or in relation to the days of tissue collection ($p > 0.05$). Conclusion: LLLT doses of 1.5 and 60 J were successful in increasing the OPD of the scar and adjacent skin.

8932-31, Session 6

Mast cellcurve response in partial partial Archilles´s tendon rupture after phototherapy 830nm

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Objective: Quantify mast cells at different time intervals after partial Archilles´s tendon rupture in rats treated with low intensity laser therapy (LLLT). Background: There is a high incidence of lesions and ruptures in the Archilles´s tendon that can take weeks and months to heal completely. Knowing that the mast cells help in the healing repair phase and that LLLT has favorable effects on this tissue repair process makes the study relevant of this modality on the quantity of mastocytes in the ruptured tendon. Sixty Wistar rats were submitted to partial Archilles´s tendon rupture by direct trauma. After the lesion, the animals were randomized into 10 groups, differentiated into the treatment group with a laser 830nm, 80mW, 1.12 J and the simulation group. Both the groups were subdivided according to the histological assessment period of the sample, either six hours, 12 hours, 24 hours, two days or three days after the rupture, to quantify the mastocytes of the tissue in question. Results: The group submitted to LLLT presented greater quantity of mastocytes in the periods of six hours, 12 hours, 24 hours, two days or three days after rupture, compared to the simulation groups but differences

were detected between the sample assessment periods only in the simulation group. Conclusion: LLLT was shown to increase the quantity of mastocytes in the assessment periods compared to the simulation groups.

8932-32, Session 7

Low-level light therapy and aesthetic dermatology *(Invited Paper)*

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Background and Motivation: Over the last few years, low-level laser (light) therapy (LLLT) has been demonstrated to be beneficial to the field of aesthetic medicine, specifically aesthetic dermatology. LLLT encompasses a broad spectrum of procedures, primarily cosmetic, which provide treatment options for a myriad of dermatological conditions. Dermatological disorders involving inflammation, acne, scars, aging and pigmentation have been investigated with the assistance of animal models and clinical trials. LLLT offers a means to address such conditions with improved efficacy versatility and no side-effects, however, comprehensive literature reports covering the utility of LLLT are scarce and thus the need for coverage arises.

Objective: A review of literature pertaining to the efficacy and applicability of LLLT for aesthetic dermatology.

Results: Recent studies suggest that LLLT may be used to treat certain cosmetically and clinically undesirable dermatological conditions such as acne vulgaris, vitiligo, herpes simplex virus infections, etc. and may aid in eliciting responses beneficial for skin appearance as in the case of light induced photo-protection and wrinkle reduction. LLLT also seems to play an influential role in procedures such as lipoplasty and liposuction, allowing for noninvasive and non-thermal methods of subcutaneous fat reduction. Finally, the most commercially successful use of LLLT is for managing alopecia in both men and women.

Conclusion: Recent studies have demonstrated the versatility of LLLT for the addressing issues in many areas of cosmetic dermatology, introducing LLLT as an effective patient-friendly medical tool which may prove to possess greater efficacy in certain aspects of aesthetic medicine. However, there are certain uncertainties regarding the exact underlying mechanisms of LLLT operating in certain proceedings and these provide opportunities for further investigation.

8932-33, Session 7

Acute effects of low-level laser therapy on gas exchange and electromyographic fatigue threshold during cardiopulmonary exercise testing in healthy adults

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Despite the positive effects of low-level laser therapy (LLLT) on muscle fatigue before exercises using single muscle group, the acute effects of LLLT on performance in cardiopulmonary exercise testing (CPET) are poorly understood. We aimed to determine the effect of LLLT before CPET on gas exchange and electromyographic response in healthy adults. A randomized double-blind placebo-controlled crossover trial was performed with 18 untrained participants (9 males; 22 ± 2 yr). The LLLT or placebo was applied to quadriceps and gastrocnemius 10 min before a rapidly incremental cycle-ergometer CPET randomly performed on alternate days. The LLLT was performed using a multi-diode cluster, 20 s/site (850 nm, 100 mW/diode, 14 J/site). Physiological responses were continuously monitored during the CPETs using a gas analyzer. The electromyographic fatigue threshold (EMGth) was assessed with surface electrodes on vastus lateralis. The root mean square (RMS) was plotted every 5 s against the exercise intensity. The EMGth was visually detected as the breakpoint in RMS values throughout the CPET. Compared to placebo, the LLLT significantly increased peak O2 uptake (V'O2: 33 ± 10 vs. 31 ± 9 mL/min/kg). We observed a shallower slope of the ?heart rate/?VO2 during the CPET after LLLT compared to placebo, i.e., increased cardiovascular efficiency (56 ± 24 vs. 66 ± 30 bpm/L/min). There were no LLLT-related changes in EMGth. We may conclude that the LLLT acutely increase exercise performance in healthy untrained adults primarily due to increased O2 extraction by peripheral muscles without causing significant impact on muscle fatigue.

8932-35, Session 7

Effect of laser acupuncture versus traditional acupuncture in neck pain of cervical spondylosis

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Aim: to compare the efficiency of laser versus traditional acupuncture as complimentary modalities in the treatment of cervical spondylosis(CS)

Material and methods: Forty female patients were randomized into two equal groups. Group A received needle acupuncture therapy according to traditional Chinese medicine with electrical stimulation for 20 min at standard at standard acupoints, ear points and Ashi point on the average 3 points.Group B received low level laser therapy (LLLT) at the same acupoints. Each points received 3 sessions /week for 4 weeks.

Results: tenderness disappeared in 65%of patients in group A and 75% of patients in group B with improved percentage in group A was 85.5% while in group B it was 89.2%. Pain on VAS related to direction of motion of 6 directions improved in all cases where of the percentage of improvement 72.46%in group A and 85.58% in group B. Pain on VAS at rest movement in all patients with percentage of improvement 80.41% In group A and 84.28% in group B. NDIQ score improvement in all patients with percentage of improvement 69.78% in group A vs 73.77% in group B. serum TNF alpha decreased in 85% of

Patients of groupA vs 95% of patients in group B. with percentage of improvement 15.95% in group A and 34.60% in group B. serum beta endorphins was increased in all patients with a percentage of improvement 12.40% in group A vs 39.67% in group B. Follow up of VAS after 6 months from the last session revealed persistent improvement in 55% of patients of group A vs 80% of patients of group B.

Conclusion: Both methods of treatment for CS gave improvement regarding pain intensity, disability and quality of life being more evident in LLLT followed for 6 month supported with improved serum TNF alpha and beta endorphin.

8932-37, Session 7

Comparisonof clinical effectiveness of laser acupuncture and amytryptalin in diabetic peripheral neuropathu (DPN): a sham controled randomized cinical trial

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Objective: To assess the effect of laser acupuncture in patients suffering from painful diabetic neuropathy and its comparison with standard of care. Patients and Method: This study was conducted in Diabetic and Endocrine Management Center (DEMC) Lahore General Hospital, Lahore, Pakistan. A randomized control trial (RCT) was opted and a total of 164 patients were chosen using Non-probability purposive sampling technique. Results: A total of 164 subjects were included in the study who were subdivided into three groups labeled as A, B and C for laser therapy treatment, amitryptaline treatment and controls respectively. The mean age of subjects was 51.54±10.46 in Group A, 49.38±10.56 in Group B and 51.70±11.43 in Group C. The difference of mean ages in all study groups was statistically insignificant (p-value= 0.469). The average pain score in patients who received laser therapy was 5.95±0.91 before treatment, whereas after treatment it was 4.31±0.98. The mean pain score in subjects having Amitryptaline before starting the treatment was 6.87±0.71 and after treatment, it was 6.23±0.98. The mean score for daily life activities in subjects who received laser therapy was 9.56±2.37 before treatment, while after treatment it was 7.56±1.54. The average score for daily life activities in patients having Amitryptaline before starting the treatment was 9.05±1.93 and after treatment, it was 8.11±1.71. Average depression and anxiety score in patients receiving laser therapy was 9.29±2.28 before treatment, whereas after treatment it was found to be 7.42±1.91. Similarly, the mean depression and anxiety score in patients of Amitryptaline group before starting the treatment was 9.38±2.21 and after treatment, it was 8.38±2.14. Conclusion: The mean score in our study reveals that laser therapy shows better outcomes in improvement of pain relief, depression, anxiety and daily life activities compared to amitryptaline in patients of diabetic neuropathy.

8932-38, Session PSun

Assessment laser phototherapy on bone defects grafted or not with biphasic synthetic micro-granular HA + ?-Tricalcium phosphate: histological study in an animal model

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Beside of biomaterials, Laser phototherapy has shown positive results as auxiliary therapy on bone repair. The aim of this study was to evaluate, through histological analysis, the influence of Laser phototherapy in the process of repair of bone defects grafted or not with Hydroxyapatite. Forty rats were divided into 4 groups each subdivided into 2 subgroups according to the time of sacrifice. Surgical bone defects were made on femur of each animal with a trephine drill. On animals of group Clot the defect was filled only by blood, on group Laser the defect filled with the clot and further irradiated. In group Biomaterial the defect was filled with HA + ß-TCP graft. In group Laser + Biomaterial, the defect was filled with biomaterial and further irradiated. The irradiation protocols were performed every 48 hours during for 15 days. Animal death occurred after 15 and 30 days. The specimens were routinely processed and evaluated by light microscopy. Qualitative analysis showed that group Laser + Biomaterial was in a more advanced stage of repair at the end of the experimental time. It was concluded that the Laser irradiation improved the repair of bone defects grafted or not.

8932-39, Session PSun

Phenothiazinium dyes in association with diode red laser against B16F10 melanoma cells: in vitro study.

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In Brazil solar incidence is high and continuous throughout the year. Body exposure to sunlight may be a key point in the rates of individuals affected by melanoma and other types of skin cancer in many countries. Brazil already occupies the 15th place in the ranking of melanoma cases and the limitations presented by drugs used in the therapy of this cancer, new approaches are being used in an attempt to decrease the mortality of this malignancy. The aim of this study was to evaluate the effects of phenothiazine compounds associated with laser light on murine melanoma (B16F10) in vitro by measuring cell growth using optical microscopy and Colorimetric Test before and after photodynamic therapy. We used a diode laser (?660nm, 2.4J/cm?, 40mW, 60s, CW) associated with phenothiazine (PTZ) (12.5 ?M, time pre-irradiation of 30 minutes). The following groups were tested: control (LF-), PTZ (L-F+), Laser (L+F-), Laser + PTZ (L+F+). The results showed a significant reduction in cell growth in the group treated by the photodynamic therapy compared to the control at 24 and 48 h ($p = 0.001$ and $p = 0.01$ respectively). Important morphological changes as vacuolization of the cytoplasm and formation of 'blebs', and nuclear changes such as chromatin condensation and marginalization were observed in group L + F +. Although the results of this study have shown a significant reduction in cell growth by appropriate combination of phenothiazine dye and laser light further studies are needed.

8932-40, Session PSun

Mechanisms of laser-induced regeneration of cartilage

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The paper presents a new approach for tissue regeneration under non-destructive laser radiation, including combine effect of low intensity laser radiation on the cells that allows changing respiratory properties of the cells and to activate them to differentiation and proliferation, and also modification of the extracellular matrix (ESM). We consider various highly interrelated processes including photochemical, thermal, thermo-mechanical, mass-transfer, and photo-destruction processes contributing to the effect of laser radiation on cartilage and bone tissues. Temporally and spatially modulated laser radiation can provide precise control of different parameters, important for the tissue modification leading to regeneration, i.e temperature, amplitude and frequency of mechanical effect, and mass transfer to and from the cells leading to an emergence of the morphogenetic gradients. Non-ablative laser radiation allows physical and chemical modifications of ECM, including development of fluctuating porous system as well as controllable replacements of interstitial water, contributing to morphogenetic gradients formation and to the developmental role of mechanical pressure and tensional loads. The results of in-vivo studies in laser repair of traumatic and post-traumatic defects in the mini-pig's joints will be presented to demonstrate the efficacy of the new approach for healing of cartilage defects and scar reduction.

8932-41, Session PSun

Evaluation of enamel by scanning electron microscopy green led associated to hydrogen peroxide 35% for dental bleaching

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Dental bleaching is a routine procedure in clinical dental practice. The literature is contradictory regarding the effects of bleaching agents on both morphology and demineralization of enamel after bleaching. The aim of this study was to analyze by SEM the effect of 35% neutral hydrogen peroxide cured by green LED. Buccal surfaces of 15 pre-molars were sectioned and marked with a central groove to allow experimental and control groups on the same specimen. For SEM, 75 electron micrographs were evaluated by tree observers at 43X, 220X and 1000X. Quantitative analysis for the determination of the surface elemental composition of the samples through X-ray microanalysis by SEM was also performed. The protocol tested neither showed significant changes in mineral composition of the samples nor to dental enamel structure when compared to controls. SEM analysis allowed inferring that there were marked morphological differences between the enamel samples highlighting the need for the use of the same tooth in comparative morphological studies. The tested protocol did not cause morphological damage the enamel surface when compared to their respective controls.

8932-42, Session PSun

Enhanced angiogenic effet of adipose-derived stromal cell spheroid with low-level laser therapy in mouse hind limb ischemia

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We investigated whether low-level laser irradiation precondition prior to adipose-derived stromal cell (ASC) spheroid transplantation improved hind limb functional recovery by the stimulation of angiogenesis and tissue regereration in a mouse hind limb ischemia. Previous reports suggested that culture as spheroids can increase the therapeutic potential of the adult stem. In in-vitro experiments, we confirmed that ASCs differentiated into endothelial cells and endothelial progenitor cell. To evaluate the therapeutic effect of ASC spheroid in vivo, PBS, human adipose tissue-derived stromal cells, and ASC spheroid were transplanted into a hind limb ischemia model. The ASC spheroid transplanted into the hind limb ischemia differentiated into endothelial cells and remained differentiated. Transplantation of ASC spheroid into the hind limb ischemia significantly elevated the density of vascular formations through angiogenic factors released by the hind limb ischemia at the lesion site, and enhanced tissue regereration at the lesion site. Consistent with these results, the transplantation of ASC spheroid significantly improved functional recovery compared with both ASC ransplantation and PBS treatment. These findings suggest that transplantation of ASC spheroid may be an effective stem cell therapy for the treatment of hind limb ischemia and peripheral vascular disease.

8932-43, Session PSun

Effect of LED phototherapy (?630 ± 20nm,) on mast cells during wound healing in hypothyroid and euthyroid rats

Gardênia M. Paraguassu, Isabele C. V. DeCastro, Rebeca M. Vasconcelos, Milena Guarda, Tânia T. Rodriguez, Maria José P. Ramalho, Antônio Luiz B. Pinheiro, Luciana Maria P. Pedreira Ramalho, Univ. Federal da Bahia (Brazil)

Hypothyroidism has been associated with the disruption of the body's metabolism, including the healing process. LED phototherapy has been studied using several healing models, but their effects on mast cells proliferation associated to hypothyroidism remains unknown. The aim of this study was to assess the effect LED (?630±20nm) phototherapy on mast cells proliferation during tissue repair in hypothyroid rats. Under general anesthesia, a standard surgical wound (1cm?) was created on the dorsum of 24 male Wistar rats divided into 4 groups of 6 animals each: EC-Control Euthyroid; ED-Euthyroid+LED; HC-Control Hypothyroid and HD-Hypothyroid+LED. The irradiation started immediately after surgery and was repeated every other day for 7 days, when animal death occurred. Hypothyroidism was induced in rats with propylthiouracil (0.05g/100mL) administered orally for 4 weeks and maintained until the end of the experiment. Toluidine blue was performed in the specimens removed for the quantitative analysis of mast cells. The mast cell proliferation was significantly higher in HC group than in EC group (Mann Whitney, $p<0.05$), but when ED group was compared to HD group, no significant difference was found. Our results showed that hypothyroidism increased mast cells population prolonging the inflammatory phase of the tissue repair and LED light had a biomodulative effect on mast cell population, even when hipothyroidism was present.

8932-44, Session PSun

Laser photobiomodulation as an adjunct of the wound healing impairment of rats exposed to a cafeteria diet

Vírginia Uzeda, Gardênia M. Paraguassu, Jean Nunes Dos Santos, Maria José P. Ramalho, Tânia T. Rodriguez, Luciana M. Pedreira Ramalho, Univ. Federal da Bahia (Brazil)

Obesity is associated to a delayed wound healing and prolonged inflammatory phase. Laser light has shown positive results in the photobiomodulation of tissue repair, however, its use associated with systemic disorders such as obesity is still little explored in the literature. The aim of this study was to validate an experimental system for studying weight gaining by consuming a high fat diet called "cafeteria diet" (CD) for the induction of obesity. Forty-eight rats were weaned, divided into two experimental groups: standard diet (SD) and Cafeteria Diet (CD). Free feeding was carried out during 20 weeks and the mass gaining was accompanied. After general anesthesia standardized surgical wounds were created (1cm ?) in the dorsal midline region of each animal. Both groups (SD; CD) were divided into 2 subgroups of 12 animals, G1 and G3 (non-irradiated) and G2 and G4 (irradiated). The irradiation protocols (?660 nm, 40 mW, CW; 24 J/cm2) started immediately after surgery and were repeated every other day during 14 days. The rats were killed at the 8th or 15th days after surgery.The abdominal fat was removed and weighed to verify the success of the induction technique. The specimens were taken and routinely processed histology (hematoxylin / eosin) was performed. It was concluded that the ingestion of fast-food increased abdominal fat in rats and modified the inflammatory pattern of the healing. Laser phototherapy in the parameters employed quickened wound healing in obese rats.

8932-45, Session PSun

LLLT and pharmacological approaches for wound-healing in cell models

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Low light level therapy (LLLT) has been used to increase wound-healing, but challenges in dosimetry and other treatment parameters have limited this approach; moreover, both positive and negative results have been reported. Using in-vitro wound healing assays, we demonstrate effectiveness of two different LLLT sources, a clinical laser (bioLITEC) and an LED array (Celluma). The Celluma emits light ranging from 465-880nm, is flexible allowing treatment of multiple wound areas, offers continuous or pulsed delivery, and is highly portable and user friendly. Most cold laser systems are large and require advanced expertise to operate. Both sources were effective at 640/652nm and 810/880nm in three different cell types: PtK2 rat kangaroo renal epithelial cells, U2OS human osteosarcoma cells and A549 adenocarcinoma human alveolar epithelial cells. The LED performed at least as well as, and, in some cases statistically better, than the clinical laser.

We have shown that a new nitric oxide donating drug nitrosyl-cobinamide (NO-Cbi) accelerates wound healing in the cell model systems described above. When LLLT is combined with NO-Cbi treatment, the rate of wound-healing is greater than with either the pharmacological or LLLT treatment alone. In conclusion, we report, (1) LED-stimulated wound healing which performs as well as a clinical laser system, and (2) a combined LLLT plus pharmacological therapy for wound-healing that out-performs either modality alone.

Conference 8933: Frontiers in Biological Detection: From Nanosensors to Systems

BiOS SPIE Photonics West

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8933 Frontiers in Biological Detection: From Nanosensors to Systems VI

8933-2, Session 1

Biosensing platform with tapered optical microfibers: new results

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Fiber sensors continue to be a topic of great interest as new techniques become available for processing and incorporating them into a measurement platform design. Due to the simplicity of coupling light in and out of the fiber, in addition to the simple and accurate fabrication methods, fiber sensors can be effective in providing quick and inexpensive analysis of analytes.

We perform both theoretical calculations and experiments on the propagation of modes through our optical fiber system. The theoretical calculations are based on modal solutions of Maxwell equations in a segmented fiber with matching boundary conditions between each segment. This provides us with accurate preliminary results on the propagation characteristics of the bi-tapered fibers and assists in the preliminary design of fiber tapers. The fabrication of a biosensor is based on the biconically tapered optical fiber which allows the evanescent light field to interact with molecules attached to the fiber surface. We use a standard optical fiber tapered to a waist diameter of approximately 7 µm. This sensing platform is capable of continuous, specific, sensitive, and label-free molecular detection in the aqueous phase. The optical modes that propagate across the tapered region create an interference pattern at the fiber output; we study antibody-antigen capture on the surface of the fiber using a wavelength tunable laser source and demonstrate that it causes shifts in the output spectrum. These shifts are consistent across multiple fiber samples, and the individual fibers are reusable. The system described above has been modified for detection of volatile organic compounds, biologically relevant analytes in the vapor phase.

8933-3, Session 1

Arrayed imaging reflectometry as a tool for multiplex label-free determination of RNA-protein binding kinetics

Benjamin L. Miller, Univ. of Rochester Medical Ctr. (United States); Amrita R. Yadav, Univ. of Rochester (United States); Charles R. Mace, Univ. of Rochester Medical Ctr. (United States)

RNA is increasingly recognized as filling several critical roles in human health and disease. To date, however, there are few technologies that address the problem of studying RNA in these roles or as a drug target in a high-throughput fashion. We report on the application of Arrayed Imaging Reflectometry (AIR) as a technique for addressing this gap in capability. AIR is a sensitive, label-free technique that reports on analyte binding via changes in the reflectivity off the surface of a silicon chip. We demonstrate that AIR is useful for understanding protein binding to RNAs implicated in Type 1 Myotonic Dystrophy.

8933-12, Session 1

Understanding and mitigating DNA induced corrosion in porous silicon based biosensors

Yiliang Zhao, Sharon M. Weiss, Jenifer L. Lawrie, Paul E. Laibinis, Vanderbilt Univ. (United States)

Porous silicon (PSi) structures have been demonstrated as effective biosensors due to their large surface area, size-selective filtering capabilities, and tunable optical properties. However, PSi surfaces are highly susceptible to oxidation and corrosion in aqueous environments and solutions containing negative charges. In DNA sensing applications, PSi corrosion can mask the DNA binding signal as the typical increase in refractive index that results from a hybridization event can be countered by the decrease in refractive index due to corrosion of the PSi matrix. Such signal ambiguity should be eliminated in practical devices. In this work, we carefully examine the influence of charge density and surface passivation on the corrosion process in PSi waveguides in order to control this process in PSi based biosensors. Both increased DNA probe density and increased target DNA concentration enhance the corrosion process, leading to an overall blueshift of the PSi waveguide resonance. While poorly passivated PSi structures have been shown to continuously degrade upon prolonged exposure to solutions containing negative charges, PSi waveguides that are sufficiently passivated to prevent oxidation/corrosion in aqueous solution exhibit a saturation effect in the corrosion process, which increases reusability of the sensor. For practical implementation of PSi DNA sensors, the negative charges from DNA must be mitigated. We show that a redshift of the PSi waveguide resonance results from either replacing the DNA probe or target with neutral charge PNA or introducing Mg2+ ions to shield the negative charges of the DNA in solution.

8933-4, Session 2

Integrated-optical waveguide and nanoparticle based label-free molecular biosensing concepts *(Invited Paper)*

Rainer Hainberger, Paul Muellner, Eva Melnik, Markus Wellenzohn, Roman Bruck, Stefan Schrittwieser, Jörg Schotter, AIT Austrian Institute of Technology GmbH (Austria); Michael Waldow, Thorsten Wahlbrink, AMO GmbH (Germany); Guenther Koppitsch, Franz Schrank, ams AG (Austria); Katerina Soulantica, Sergio Lentijo, Institut National des Sciences Appliquées de Toulouse (France); Beatriz Pelaz, Wolfgang Parak, Philipps-Univ. Marburg (Germany)

Label-free optical schemes for molecular biosensing have a strong potential for a wide range of applications in medical research and diagnostics. Apart from analytical requirements such as sensitivity, specificity, and multiplexing capability, also other aspects such as ease of use and manufacturability have to be considered in order to facilitate their practical implementation.

In this presentation, we present silicon and polymer photonic as well as magnetic nanoparticle based molecular biosensor concepts that address these aspects.

With respect to integrated optical waveguide devices, evanescent wave sensing by means of Mach Zehnder interferometers are used as biosensing components. We pursue three approaches: a) silicon photonic wire waveguides enabling on-chip wavelength division multiplexing and utilization of slow light in photonic crystal defect waveguides operated in the 1,3 µm wavelength regime, b) silicon nitride photonics wire waveguide devices compatible with on-chip photodiode integration operated in the 0,85 µm wavelength regime, and c) spin-coated polyimide waveguide devices for cost-effective production operated in the 1,3 µm wavelength regime.

The nanoparticle based concept relies on a plasmon-optical detection of the hydrodynamic properties of magnetic-core/gold-shell nanorods immersed in the sample solution. The hybrid nanorods are rotated

within an externally applied magnetic field and their rotation optically monitored. When target molecules bind to the surfaces of the nanorods their hydrodynamic volumes increase, which directly translates into a change of the optical signal. This approach possesses the potential to enable real-time measurements with only minimal sample preparation requirements, thus presenting a promising point-of-care diagnostic system.

8933-5, Session 2

Suspended micro-ring resonator for enhanced biomolecule detection sensitivity

Shuren Hu, Kun Qin, Vanderbilt Univ. (United States); Ivan I. Kravchenko, Scott T. Retterer, Oak Ridge National Lab. (United States); Sharon M. Weiss, Vanderbilt Univ. (United States)

Silicon micro-ring biosensors are highly attractive for high sensitivity and multiplexed Lab-on-Chip systems. Here, we characterize the sensing performance of suspended TM-mode silicon micro-ring resonators, 5 ?m in radius, and demonstrate an enhanced sensitivity to molecular binding on the ring after suspension. In the TM-mode, the overall field intensity exists primarily outside of the waveguide core, with high electric field intensities present near the top and bottom surfaces. For traditional micro-ring resonators, only the top surface of the ring is available for surface analyte attachment, with the electric field intensity near the bottom surface leaking into the SiO2 substrate. In our approach, we suspend the TM-micro ring resonators in order to not only increase the surface area for binding events and increase the light-matter interaction with analytes but also to enable higher quality factor micro-rings, which will further reduce the detection limit of bound analytes. Six supporting trusses, 1 ?m long, 100 nm x 270 nm in width and height are used to support the TM ring resonators; the underlying SiO2 substrate is wet etched in HF. The suspended rings demonstrate excellent mechanical stability to multiple rinsing, soaking and nitrogen drying steps during the sensing procedure. We show that the resonance shift achieved by the suspended rings after attachment of small chemical molecules and DNA is at least twice that of micro-rings supported by the SiO2 substrate.

8933-6, Session 2

Highly sensitive integrated optical biosensors

Alethea V. Zamora Gomez, Peter Luetzow, Martin Weiland, Daniel Pergande, Fraunhofer-Institut für Nachrichtentechnik Heinrich-Hertz-Institut (Germany); Henning Schroeder, Fraunhofer Institute for Reliability and Microintegration (Germany)

Optical sensor systems for biological and medical applications have been widely developed in order to satisfy the current requirements such as a miniaturization, cost reduction, label-free detection and fast response. Here, we demonstrate a highly sensitive optical sensor based on two cascaded microring resonators (MRRs) exploiting the Vernier effect. The architecture consists of a filter MRR connected to a sensor MRR via a common waveguide. The external medium of the filter MRR is isolated with a top cladding layer, while the sensor MRR interacts with the analyte sample via an opening. The sensor chip, that includes an array of five cascaded MRRs, was designed and fabricated on a silicon nitride platform. A first test has been performed with sodium chloride (NaCl) concentrations in deionized (DI) water providing a sensitivity of 1.03 nm/% (6317 nm/RIU). A limit of detection of 3.16 x 10-6 RIU was demonstrated for the current sensor, respectively. Several concentrations of isopropanol in ethanol ranging from 0% to 10% were also investigated. These preliminary measurements show a sensitivity as high as 0.95 nm/% at ~1535 nm compared to 0.02 nm/% from a single sensor MRR. For a moderated alignment between the chip and cleaved optical fibers, tapered grating couplers are included at the ends of waveguides. Hence, by combining the Vernier effect and the silicon nitride material, cascaded MRRs will be a powerful optical configuration for biosensing applications in a wide operating wavelength range.

8933-7, Session 2

Detection of target DNA using photo-reactive protoporphyrin moeity on a nanocomposite substrate

Sumana Das, Madhusmita Mishra, Ramakrishna Vasireddi, D. Roy Mahapatra, Indian Institute of Science (India)

Detection of pathogens from infected biological samples through conventional process involves cell lysis and purification. The main objective of this work is to minimize the time and sample loss, as well as to increase the efficiency of detection of biomolecules. Electrical lysis of medical sample is performed in a closed microfluidic channel in a single integrated platform where the downstream analysis of the sample is possible. The device functions involve, in a sequence, flow of lysate from lysis chamber passed through a thermal denaturation counter where dsDNA is denatured to ssDNA, which is controlled by heater unit. A functionalized binding chamber of ssDNA is prepared by using ZnO nanorods as the matrix and functionalized with bifunctional carboxylic acid, 16-(2-pyridyldithiol) hexadecanoic acid (PDHA) which is further attached to a linker molecule 1-ethyl-3-(3-dimethylaminopropyl) (EDC). Linker moeity is then covalently bound to photoreactive protoporphyrin (PPP) molecule. The photolabile molecule protoporphyrin interacts with -NH2 labeled single stranded DNA (ssDNA) which thus acts as a probe to detect complimentary ssDNA from target organisms. Thereafter the bound DNA with protoporphyrin is exposed to an LED of particular wavelength for a definite period of time and DNA was eluted and analyzed. UV/Vis spectroscopic analysis at 260/280 nm wavelength confirms the purity and peak at 260 nm is reconfirmed for the elution of target DNA. Quantitative and qualitative data obtained from the current experiments show highly selective detection of biomolecule such as DNA which have large number of future applications in Point-of-Care devices.

8933-8, Session 3

Fluorescent nanosensors for monitoring neurotransmitter release *(Invited Paper)*

Heather A. Clark, Ryan Walsh, Jennifer Morales, Northeastern Univ. (United States)

What are thought and consciousness? From a physiological perspective, thoughts are a set of electrical and chemical signals that control memory, emotion, and cognition through specific neural circuits. Current techniques for measuring neuronal signals and activity are limited to calcium and voltage signaling, point measurements such as electrodes and microdialysis or general function such as fMRI. These techniques have uncovered a wealth of information, but techniques for imaging real-time, spatially relevant, and chemically specific information are still limited. We have just recently established a modular system to couple various enzymes and readout mechanisms. Specifically, we have developed nanosensors to detect two target analytes: acetylcholine and dopamine. The core of the sensor utilizes an enzyme that metabolizes the target, and an accompanying nanosensor that selectively responds to the enzymatic reaction product. Although this work will focus on two neurotransmitters, in principle this approach is easily expanded to any desired target that has an enzyme that generates a measurable product. The scaffold of the sensor is composed of DNA dendrimers with the recognition units covalently attached. The sensors have demonstrated a dynamic response in a physiologically relevant range, reversibility, and fast response times. The sensors will enable us to image neurotransmitter release or biochemical signaling inside and between cells and will begin to integrate the chemical processes of neurobiology with memory and brain function and answer the chemical question: What is in a thought?

8933-9, Session 3

Label-free assay for the detection of glucose mediated by the effects of narrowband absorption on quantum dot photoluminescence

Saara A. Khan, Gennifer T. Smith, Audrey Ellerbee, Stanford Univ. (United States)

We present a novel system for label-free detection of glucose based on CdSe/ZnS core/shell quantum dots (QDs). We uniquely exploit the concentration-dependent, narrowband absorption of the hexokinase-glucose 6-phosphate dehydrogenase enzymatic assay to selectively filter a 365-nm excitation source, leading to a proportional decrease in the photoluminescence intensity of the QDs. The visible wavelength emission of the QDs enables quantitative readout using standard visible detectors (e.g., CCD). Experimental results show highly linear QD photoluminescence over the clinically relevant glucose concentration range of 0.56-30mM, in excellent agreement with detection methods demonstrated by others. The method has a demonstrated limit of detection of 3.5?M, also on par with the best proposed methods.

A significant advantage of our strategy is the complete elimination of QDs as a consumable. In contrast with other methods of QD-based measurement of glucose, our system does not require the glucose solution to be mixed with the QDs, thereby decreasing its overall cost and making it an ideal strategy for point-of-care detection of glucose in low-resource areas. Furthermore, readout can be accomplished with low-cost, portable detectors such as cellular phones, eliminating the need for expensive and bulky spectrophotometers to output quantitative information. The general strategy we present is useful for other biosensing applications involving chemistries with unique absorption peaks falling within the excitation band of all available QDs.

8933-10, Session 3

Utilizing embedded optofluidic sensors for flourescent detection measurements in space and time

Mark C. Harrison, Andrea M. Armani, The Univ. of Southern California (United States)

The field of biodetection has been significantly impacted by integrated waveguide biosensors, especially when combined with fluorescent labeling. While there are numerous types of fluorescent waveguide sensors, many rely on the evanescent filed to excite fluorophores. The light emitted from the fluorophores is detected either directly via imaging above the waveguide or indirectly via a decrease in power transfer. Recently, a sensor device which back-couples emitted fluorescent light into the waveguide was experimentally demonstrated. This allows the fluorescent signal to be detected directly. While previous experimental work allowed the development of an empirical model, it did not develop a rigorous theoretical model. Additionally, the previous work focused on performing detection using the novel back-coupling route without comparing the efficiency of detection with the more conventional imaging technique. In order to predict the device's sensing performance in both air and aqueous environments, in the present work we develop finite difference time domain simulations, enabling the optimization of the device for either biological or aerosol detection. We also verify the modeling with complementary experiments, measuring the fluorescence coupled into the waveguide and radiated perpendicular to the waveguide, allowing direct comparison between these two detection routes. Finally, we utilized spatiotemporal measurements of the fluorescence on the waveguide to measure the fluorescent decay rate of the fluorescent dye at arbitrary points along the length of the waveguide. More in-depth characterization of the preferential coupling phenomenon, as well as these unique spatiotemporal measurements will be useful to the development of future optical biosensing devices.

8933-1, Session 4

Dead/alive bacteria detection using an all-fibre optical system

Evgeny Bogomolny, Michael Cheng, Simon Swift, Frédérique Vanholsbeeck, The Univ. of Auckland (New Zealand)

In recent years, the cyclic dinucleotide cyclic diguanylate (c-di-GMP) has emerged as a prominent messenger that coordinates the cellular functions associated with bacterial biofilm formation and pathogenicity.

In the present study, we have utilised genetically encoded fluorescent biosensors for monitoring cellular c-di-GMP concentrations under different growth conditions for uropathogenic Escherichia coli (UPEC). FRET derived from the standard cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) biosensors linked by a c-di-GMP binding domain is inactivated by conformational change induced by binding to c-di-GMP. The intracellular level of c-di-GMP is reflected by the ratio of cyan:yellow fluorescence.

To measure fluorescence signal we have exploited an all-fibre optical system using stable DPSS lasers and a sensitive CCD-based spectrometer. The laser shutter controlled allows the sample exposure time to be synchronized with the acquisition time of the spectrometer to minimize the effect of photobleaching. Recently, we have designed an efficient dual-fibre probe that increases the detection sensitivity up to 1 colony forming unit/ml.

Our results clearly enable non destructive and in-situ differentiation between aggregating (iron restricted, high c-di-GMP) and dispersing (iron replete, low c-di-GMP) populations of UPEC. For example, we have observed significant FRET incensement during iron-induced dispersal when compared to aggregating cells. In addition, results obtained with the all-fibre optical system are corroborated using standard fluorescence spectroscopy. As FRET-based sensors become prevalent in diverse applications in biological and medical research there is an increasing need for higher fluorescence sensitivity and improved specificity to which our optical system may offer a robust solution.

8933-11, Session 4

Förster (fluorescence) resonance energy transfer (FRET) activated by biosensors: study of bacteria cellular activity of cyclic diguanylate using an advanced all-fibre optical system

Evgeny Bogomolny, Frédérique Vanholsbeeck, Michael Cheng, Guneet Kaur, Simon Swift, The Univ. of Auckland (New Zealand)

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8933-13, Session 4

Photonic crystal enhancement of a homogeneous fluorescent assay using submicron fluid channels fabricated by e-jet patterning

Yafang Tan, Erick Sutanto, Andrew G. Alleyne, Brian T. Cunningham, Univ. of Illinois at Urbana-Champaign (United States)

We demonstrate the enhancement of a liquid-based homogenous fluorescence assay using the resonant electric fields from a photonic crystal (PC) surface. Because evanescent fields are confined to the liquid volume nearest to the photonic crystal, we developed a simple approach for integrating a PC fabricated on a silicon substrate within a fluid channel with submicron height, using electrohydrodynamic jet (e-jet) printing of a light-curable epoxy adhesive to define the fluid channel pattern. The PC is excited by a custom-designed compact instrument that illuminates the PC with collimated light that precisely matches the resonant coupling condition when the PC is covered with aqueous media. Using a molecular beacon nucleic acid fluorescence resonant energy transfer (FRET) probe for a specific miRNA sequence, we demonstrate an 8x enhancement of the fluorescence emission signal, compared to performing the same assay without exciting resonance in the PC detecting a miRNA sequence at a concentration of 62nM from a liquid volume of only ~20 nl. The approach may be utilized for any liquid-based fluorescence assay for applications in point-of-care diagnostics, environmental monitoring, or pathogen detection.

8933-14, Session 4

Multiplexed detection of breast cancer cell lysates in silicon photonic crystal microcavity biosensors

Liang Zhu, The Univ. of Texas at Austin (United States); Swapnajit Chakravarty, Omega Optics, Inc. (United States); Wei-Cheng Lai, Yi Zou, Ray T. Chen, The Univ. of Texas at Austin (United States)

High throughput multiplexed chip-based label-free biosensors are important for several applications ranging from medicine and drug discovery to food pathogen detection, environmental pollution detection and biodefense. In medical research, where patient samples are available to a limited number of research labs, significant benefits are available with the ability to multiplex several specific and control binding affinity studies of several candidate biomarkers with a small volume of sample. Chip-based silicon photonics has the potential to serve this need from the perspective of both cost and performance. At the same time, chip based multiplexing offers the potential to miniaturize components to portable assays that can be used in remote, low-resource settings. We have previously demonstrated that biosensors comprising a photonic crystal (PC) microcavity coupled to a photonic crystal waveguide show sensitivities down to concentrations of 50fM (3pg/ml). We also showed experimentally that lung cancer cell line lysates can be detected with high sensitivity and specificity with multiplexed PC microcavity biosensors. In this paper, we experimentally detect breast cancer cell line lysates via a 16-plexed label-free silicon based L13 PC microcavity sensors. A two-stage cascaded 1?4 multimode interference (MMI) power splitter distributes the input light from a single optical input via a sub-wavelength grating coupler. Specific and control antibodies are ink-jet printed on individual microcavities. All antibodies are printed in duplicate for statistical confidence. All sensors are measured simultaneously at the same instant of time. Sensitivity data and label free specificity via multiplexed sensing experiments will be demonstrated.

8933-15, Session 4

Measurements of affinity and dissociation constants in silicon based high sensitivity photonic crystal microcavity biosensors

Dakota Crisp, Southeast Missouri State Univ. (United States); Swapnajit Chakravarty, Omega Optics, Inc. (United States); Wei-Cheng Lai, Liang Zhu, Ray T. Chen, The Univ. of Texas at Austin (United States)

Photonic crystal (PC) microcavities have demonstrated the highest sensitivities among label free chip based optical biosensors. Sensing of different kinds of biomolecules by multiplexing PC microcavities, together with specificity via sandwich assay methods, has also been demonstrated. However, studies on the measurement of biomolecular affinity in these PC structures are limited. The study of affinity constants has tremendous implications in biomarker discovery and drug discovery for the pharmaceutical industry. Recent reports in the literature in zeptomolar sensitivity PC nanolaser biosensors have measured affinities several orders of magnitude larger between a probe biomolecule and its specific conjugate target. However, the results appear to be in error since the affinity between a probe and its specific conjugate target biomolecule is determined by the association constant (Ka) and/or binding affinity (?G) of the biomolecules and not by higher or lower sensitivity of the corresponding functionalized photonic crystal (PC) micro-cavities. In this paper, we demonstrate the methodology of calculating Ka and ?G based off of experimental data received from L13 and L55 silicon based PC micro-cavities that have previously demonstrated sensitivities down to 50femto-molar. Using the net resonance wavelength shifts from multiple ligand concentrations provides a more holistic approach of characterizing binding kinetics rather than extracting the affinity from a single concentration. Net resonance wavelength shifts are collected from complete association and dissociation concentration cycles. Experimental results will be presented.

8933-16, Session 5

Integrating SPR-ellipsometry and electrochemical measurements for performance evaluation of label-free thiophene-based biosensor

P. I. Tsai, C. K. Lee, National Taiwan Univ. (Taiwan); S. S. Lee, National Taiwan Ocean Univ. (Taiwan); S. T. Chou, National Taiwan Univ. (Taiwan); Y. T. Chang, A. S. Y. Lee, Tamkang Univ. (Taiwan)

Copuing the surface plasmon resonance (SPR) reflectance changes with a circularly polarized ellipsometry and an electrochemical impedance spectroscopy (EIS) were identified to be able to characterize the critical roles of biomolecules for vastly different biological functions and processes. Throughout the course of this study, interferon-gamma (IFN-?) was chosen as the biomarker to test and to verify the performance of this newly developed system for Tuberculosis (TB) detection. The interactions of IFN-? with immobilized anti-IFN-? antibody at various concentrations were interrogated both optically and electrochemically. A conductive

linker bis-thiophene was thiolated to ensure the cross-linked monoclonal human IFN-? antibody got self-assembled onto the gold thin film and form a label-free biosensor.

The functional features of the bis-thiophene coated-gold film were characterized by cyclic voltammetry and impedance spectroscopy methods. The association of IFN-? to the bis-thiophene bridging units via antibody-antigen interactions provided the basis for ultrasensitive detection of IFN-? by tracking the conformational changes in surface-bound protein molecules. The phase shift can be attributed to the average thickness and the real-time index of refraction of the protein layer in different protein layer. Experimental results obtained by impedance spectroscopy and by phase-interrogation SPR showed linear dynamic range. Our experimental results verified that an increase in the concentration of the IFN-? usually accompanied by phase increase in SPR and an impedance decrease in EIS. These results indicated that our newly developed integrated biosensing system can potentially provide important new insight into various conjugate phenomena and interfacial processes for observing molecular conformational changes.

8933-17, Session 5

Bowtie plasmonic nanoantenna arrays for polarimetric optical biosensing

Jonathan Calderón, Jesús Álvarez, Juan P. Martinez-Pastor, Daniel Hill, Univ. de València (Spain)

Metal nanostructures hold great potential for the realization of highly sensitive optical biosensors as they present a localized surface plasmon resonance (LSPR) which is very sensitive to their size, shape and surrounding refractive index. When two of these structures are close enough for strong coupling to take place between them, intense electromagnetic fields result within the gap. Subsequent refractive index variations in the gap results in higher resonance peak displacements than those from non-coupled metal nanostructures.

In the work presented we report on a polarimetric plasmonic biosensor based on a bowtie nanoantenna array acting as the transducer in combination with a high resolution polarimetric readout platform. The bowtie nanoantenna array has the property of not only presenting a localized surface plasmon resonance but also, due to its asymmetrical design, it produces a phase retardation between the light components polarized along the parallel and perpendicular direction respect to the axis of the nanoantennas. Using the Finite Element Method (FEM) this phase retardation was calculated as a function of the design parameters of the bowtie array. By changing the refractive index of the top cladding of the nanoantenna arrays a bulk sensitivity around 10 rad/RIU was obtained, which results in detection limits ~1E-7 refractive index units. Lastly, as analyte capture takes places within the first 10-20nm from the surface of the nanoantennas where the electromagnetic field is enhanced, the surface sensitivity of the nanoantennas array was studied by simulating the coverage of bioreceptor and analyte layers.

8933-18, Session 5

Label-free optical sensing on hybrid plasmonic-nanobiosilica platforms

Fanghui Ren, Jeremy Campbell, Gregory L. Rorrer, Alan X. Wang, Oregon State Univ. (United States)

Inspired by advanced nanofabrication techniques, rationally designed plasmonic sensors have gained tremendous research interest in recent years. Although unprecedented sensitivity, repeatability and specificity have been achieved, many of these rationally designed plasmonic sensors are manufactured using cost-prohibitive, top-down nanofabrication techniques and may not be feasibly used as disposable sensors for point-of-care applications. Diatoms are photosynthetic micro-organisms that create their own skeletal shells of hydrated amorphous silica, called frustules, which naturally possess hierarchical micro- & nano-scale features that are extremely cumbersome and expensive to duplicate by top-down fabrication techniques. These diatom nanostructures are readily and cheaply obtained by cultivation of diatom cells, followed by isolation of the diatom frustules through conventional chemical separation techniques. In this paper, we report that strong plasmonic resonances associated with hybrid biological-plasmonic nanostructures, i.e. the diatom frustule and the self-assembled nanoparticles, can simultaneously obtain ultra-high sensitivity and molecular structural resolution for various biomolecules, which will be detected by RI and SERS sensing. Specifically, Raman signals correlate to signature chemical bonds within the biomolecules, for example, the 1645cm-1 peak corresponds to amide groups. Thus it is possible to probe the bound molecule for structural information to discriminate between specific and non-specific binding. Furthermore, the reactive silanol groups on the diatom biosilica surface are also readily functionalized to attach biomolecules, such as antibodies, to the diatom frustule, which will significantly improve the molecular binding capability.

8933-19, Session 5

Ultrasensitive DNA hybridization monitoring on nanoporous gold disks in microfluidics

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Label-free sensing of trace biomolecules such as DNA and pathogens such as viruses would enable powerful amplification-free biosensing. Surface-enhanced Raman spectroscopy (SERS) has been widely used for molecular detection and identification by exploiting the localized surface plasmon resonance effect when the target molecules are near gold or silver nanostructures. However, effective and robust SERS assays have yet become a reality for trace detection.

Recently, we have developed a SERS substrate by shaping nanoporous gold thin films into monolithic submicron disks, called nanoporous gold (NPG) disks. NPG disks provides an effective surface area >10X larger than its geometrical area and a SERS enhancement factor larger than 100 million [1]. Our approach features hybrid fabrication by combining top-down planar large-area sputter etching and bottom-up atomic self assembly during dealloying. The resulted structure is thus hierarchical with the external disk shape and the internal porous network. We have selected 785 nm as the laser excitation wavelength and benzenethiol (BT) molecules as the SERS marker since the absence of a BT absorption peak near 785 nm minimizes the ambiguity presented by resonant Raman scattering, while the ability of BT to form self-assembled monolayers (SAMs) enables the number of molecules on individual NPG disks to be quantified. Additionally, the SERS activity at 785 nm laser excitation has critical significance for deep tissue penetration in any potential biomedical applications.

NPG disk substrates have been employed in SERS label-free biosensing. We have demonstrated the robust SERS measurement of 1 nM target in a microfluidic environment, which is orders of magnitude lower compared to existing work.

References

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8933-20, Session 6

Imaging magnetometer for bio-sensing based on nitrogen-vacancy centers in diamond

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We present a widefield microscopy system for imaging super-paramagnetic nanoparticles (SPNs), and propose to use it as a bio-sensing system wherein SPNs are used as tags. Potential advantages of magnetic tags over conventional fluorescent tags include the elimination of noise from auto-fluorescence, optical isolation of the biological system from the measurement apparatus, and the potential for magnetic removal of non-specifically bound material. The microscope magnetic sensing surface is composed of a thin layer of nitrogen-vacancy defect centers in the top 200 nm of a diamond substrate. Nitrogen-vacancy centers in diamond have been shown to be suitable for use as highly sensitive magnetometers due to their long spin-coherence time at room temperature. Furthermore, spin-dependent photoluminescence allows for simple far-field optical readout of the spin state, which in turn allows for optically-detected magnetic resonance measurements. We will present our results detecting single, lithographically defined (60 nm)3 iron nanoparticles. With the current sensitivity of 9 μT?Hz-1/2, we expect to be able to detect single (20 nm)3 magnetite SPM's, our proposed tags, in less than one minute. By further optimizing the sensor surface, we predict DC magnetic sensitivities below 1 μT?Hz-1/2. We will report on improvements in sensitivity and progress toward imaging fields from single magnetite SPNs.

8933-21, Session 6

Smart-phone based albumin testing in urine

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Chronic kidney disease is a major public health concern. The adverse outcomes of kidney diseases can be prevented or delayed by early detection and treatment. As part of routine health screening tests performed in hospitals, albumin testing in urine is used to diagnose early stages of kidney diseases. Since albumin levels fluctuate in urine, timed collection or testing methods are performed every few hours through the use of bulky benchtop urine analyzers, which require successive patient visits to e.g., central clinics.

To provide a field-portable and potentially home-based solution to this problem of frequent urine testing, here we introduce a personalized digital sensing platform, termed Albumin Tester, running on a smart-phone that images and automatically analyses fluorescent assays confined within disposable test tubes toward sensitive and specific detection of albumin in urine. Employing a mechanical attachment installed on the camera unit of a smart-phone, the test and control tubes are excited by a laser diode. The cellphone camera captures the fluorescent images of these tubes using a plastic external lens. The acquired cellphone images are then rapidly (within < 1sec) processed using an Android application running on the same cellphone for the quantification of albumin concentration in urine. Based on a simple sample preparation step taking ~ 5min, we experimentally confirmed the detection limit of this platform as ~ 5-10 ug/mL in buffer and synthetic urine samples. This smart-phone based albumin-testing tool could impact early diagnosis of kidney problems or monitoring of chronic patients.

8933-22, Session 6

Dual-mode lensless imaging device for digital enzyme linked immunosorbent assay

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Digital enzyme linked immunosorbent assay (ELISA) is an ultra-sensitive technology for detecting biomarkers and viruses etc. As a conventional ELISA technique, a target molecule is bonded to an antibody with an enzyme by antigen-antibody reaction. In this technology, a femto-liter droplet chamber array is used as reaction chambers. Due to its small volume, the concentration of fluorescent product by single enzyme can be sufficient for detection by a fluorescent microscopy.

In this work, we demonstrate a miniaturized lensless imaging device for digital ELISA by using a custom image sensor. The pixel array of the sensor is coated with a 20 ?m-thick yellow filter to eliminate excitation light at 470 nm and covered by a fiber optic plate (FOP) to protect the sensor without resolution degradation. The droplet chamber array formed on a 50?m-thick glass plate is directly placed on the FOP.

In the digital ELISA, microbeads coated with antibody are loaded into the droplet chamber array, and the ratio of the fluorescent to the non-fluorescent chambers with the beads are observed. In the fluorescence imaging, the spatial resolution is degraded by the spreading through the glass plate because the fluorescence is irradiated omnidirectionally. This degradation is compensated by image processing and the resolution of ~35 ?m was achieved. In the bright field imaging, the projected images of the beads with collimated illumination are observed. By varying the incident angle and image composition, microbeads were successfully imaged.

8933-23, Session 6

Molecular analysis and imaging at the nanoscale by soft x-ray laser ablation mass spectrometry

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We have pioneered the use of bright soft x-ray (SXR) laser beams for nanoscale ablation. These experiments in combination with mass analysis of the ions in the laser ablation plume, have allowed us to demonstrate, for the first time, 3D SXR mass spectrometry imaging at the nanoscale. In this paper we will report results on the molecular analysis and composition mapping of bio/organic samples by SXR laser ablation mass spectrometry.

Soft x-ray laser ablation mass spectrometry exploits the high focusibility and the distinct interaction of a 46.9 nm wavelength laser beam, from a unique compact discharge-driven SXR laser, to ablate analyte volumes of a few atto-liter. We will show the method detects intact molecular ions from aminoacids and antibiotics with mass of several hundred Da. We will also present results of 3D chemical imaging with image-voxel size down to ~2 atto-liters. These images show the molecular and elemental distribution of heterogeneous samples containing metal, dielectric and polymer patterns and of metallic nanoparticles distributed within an organic matrix with high spatial resolution, sensitivity and molecular specificity. These proof-of-principle experiments are opening exciting possibilities for mapping the chemical composition of single cells with

unprecedented spatial resolution and sensitivity.

Work supported by NIH/NIAID and by the National Science Foundation through Award EEC-0310717

8933-24, Session PSun

Detection of campylobacter jejuni using whispering gallery mode optical biosensors in an aqueous environment

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Campylobacter jejuni causes over two million cases of gastrointestinal infections, costing as much as $4.2 billion in the United States alone. Current detection methods for Campylobacter jejuni (i.e. ELISA, culture, and other amplification methods) are of limited use due to intensive and time consuming protocols that require highly skilled technicians. Developing improved methods to monitor Camplobacter jejuni in wastewater is important for safety and the prevention of food-borne illness. Our method utilizes Whispering Gallery Mode (WGM) optical resonators as novel, label-free biosensors to report high sensitivity and specificity in real-time using very low concentrations of target antigen. The surface of silica microspheres is selectively functionalized with the antibody species, Anti-Campylobacter. The target antigen, heat-killed Campylobacter jejuni ATCC 43477, is then injected into a flow chamber confirming detection. High sensitivity is monitored at each step of production by testing its Quality Factor. The surface coverage is evaluated using X-ray photoelectron spectroscopy and fluorescence microscopy. Confirming this technique extends the abilities of WGM resonators to perform as biosensors in real-world scenarios.

Conference 8934: Optical Coherence Tomography and Coherence Domain Optical Methods in Biomedicine XVIII

BiOS SPIE Photonics West

Monday - Wednesday 3 –5 February 2014

Part of Proceedings of SPIE Vol. 8934 Optical Coherence Tomography and Coherence Domain Optical Methods in Biomedicine XVIII

8934-1, Session 1

Recent Advances in MEMS-VCSELs for High Performance Structural and Functional SS-OCT Imaging

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This paper describes recent advances in MEMS-VCSEL technology for high performance structural and functional swept-source OCT imaging. These results encompass advances in both semiconductor device development and OCT imaging/system development. In particular, we report the first electrically pumped 1050nm MEMS-VCSEL sources with tuning range appropriate for ophthalmic imaging, achieving 67nm tuning bandwidth and >1mW pre-amplified optical power, corresponding to ~40mW amplified power. Like previous optically pumped devices, these devices employ electrostatic actuation in a MEMS structure to contract a variable air gap and tune laser wavelength. As before, the short VCSEL cavity and low MEMS mirror mass enable wide-range single-longitudinal-mode tuning at very high scan rates. Electrically pumped results approach the performance of previous optically pumped devices, but at substantially reduced cost and complexity. In addition, device tuning is achieved at up to 890kHz repetition rates, suggesting the possibility of axial scan rates beyond 1.7MHz without optical buffering. We also report high-gain, wideband 1050nm semiconductor optical amplifiers, which enable 40mW amplified MEMS-VCSEL power with a single amplification stage, and have enabled advances in structural and functional ophthalmic imaging. Using these advances, along with progress in 1310nm MEMS-VCSEL OCT imaging, we show representative imaging encompassing wide-field retinal imaging and dye-free OCT angiography at 1050nm, along with micromotor-based endoscopic imaging of the esophagus at 1310nm. Finally, we extend previous detection-limited measurements of MEMS-VCSEL coherence length out beyond 10 meters using a fiber-based system to introduce long delays.

8934-2, Session 1

Akinetic all-semiconductor programmable swept-source at 1550 nm and 1310 nm with centimeters coherence length

Marco Bonesi, Medizinische Univ. Wien (Austria); Michael P. Minneman, Jason Ensher, Insight Photonic Solutions, Inc. (United States); Behrooz Zabihian, Harald Sattmann, Medizinische Univ. Wien (Austria); Paul Boschert, Erich Hoover, Michael Crawford, Insight Photonic Solutions, Inc. (United States); Wolfgang Drexler, Medizinische Univ. Wien (Austria)

We demonstrate, for the first time, OCT imaging capabilities of a novel, akinetic (without any form of movement in the tuning mechanism), all-semiconductor, all-electronic tunable, compact and flexible swept source laser technology at 1550 nm and 1310 nm. To investigate laser sources performances, 2-D and 3-D ex vivo and in vivo OCT imaging was performed at different sweep rates, from 20 kHz up to 200 kHz, with different axial resolutions, about 10 µm to 20 µm, and at different coherence gate displacements, from zero delay to >17 cm

8934-3, Session 1

High-speed wideband and long coherence length swept sources for Optical Coherence Tomography

Brian D. Goldberg, Peter Whitney, Mark Kuznetsov, Walid Atia, Bart C. Johnson, Ranko Galeb, Vaibhav Mathur, Randy Murdza, Dale Flanders, AXSUN Technologies Inc. (United States)

Typical swept-source lasers for Optical Coherence Tomography (OCT) have tuning bandwidths on the order of 100nm. While this is sufficient for many clinical applications, other OCT applications including endoscopic, skin, and dental OCT would benefit from broader bandwidths and the resulting higher axial resolution. We present newly developed wideband swept-source lasers for OCT with over 140nm tuning bandwidth centered at 1310nm at both 50 and 100kHz A-line rates. The combination of high tuning rate and broad tuning bandwidth is an important step in enabling the next generation of high-performance clinical OCT systems. In addition, we present a long coherence length swept laser at 1050nm ideal for extended depth imaging

8934-4, Session 1

Region of interest based digital adaptive optics for anisotropic aberration in high resolution full field swept source OCT

Abhishek Kumar, Wolfgang Drexler, Rainer A. Leitgeb, Medizinische Univ. Wien (Austria)

The assumption of isotropic aberration across the pupil plane or, in other words, shift invariant point-spread-function (PSF) may not be valid for optical systems with higher numerical aperture (NA). Also,the lateral inhomogeneity in the sample may also cause spatially varying PSF or in general anisotropic aberrations across the field of view (FOV). We demonstrate the proof of principle of region of interest (ROI) based phase correction, using a novel non iterative digital adaptive optics based on sub-aperture correlation, for spatially varying aberration present across an enface image at a given depth location in the sample when using high NA microscope objective (MO) in a full field swept source OCT setup. We are able to achieve diffraction limited images of a layer of microspheres of size ~ 1 micron placed 1.1 mm outside the focal plane of the MO with NA of 0.65. The results show that in future it may be possible to achieve 3-D OCT images with ultra-high lateral resolution with extended depth of focus without using any external hardware or adding any system complexity. The ROI based phase correction approach also opens up the possibility to characterize the spatially varying aberrations of a MO with high NA at different defocus planes.

8934-5, Session 1

Focus-extension by depth-encoded synthetic aperture in Optical Coherence Tomography

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We present a novel method to extend the depth-of-focus of Optical Coherence Tomography (OCT). OCT is an interferometric imaging technique that provides depth-resolved scattering information. The axial resolution in OCT is provided by the coherence gate and is invariant over the full image depth. The lateral resolution is determined by the beam parameters such as wavelength and numerical aperture. The Rayleigh range determines the depth range over which the lateral resolution can be maintained. The lateral resolution is often sacrificed to maintain relatively long Rayleigh range. In this study, we propose to use a depth-encoded synthetic aperture detection scheme to extend the depth range over which a sharp focus can be maintained beyond the Rayleigh range. An annular phase plate is inserted into the light path in the sample arm, which gives rise to three separate images in a single B-scan, corresponding to three different optical path length encoded apertures. These three images are coherently summed after phase-manipulation to reconstruct a new image with a lateral resolution that is maintained over a five times larger depth range.

8934-6, Session 1

Extended depth-of-focus OCT imaging using energy-efficient low-Fresnel-number Bessel-like beams

Dirk Lorenser, C. Christian Singe, Andrea Curatolo, Sebastian R. Henn, David D. Sampson, The Univ. of Western Australia (Australia)

The use of axicon-like optical elements in a double-pass configuration is normally avoided in OCT imaging systems due to the large sensitivity penalty that results from the typically very small fraction of power in the central lobe of Bessel-like beams. For applications that call for an extended depth of focus (DOF) and where simplicity or compactness preclude the use of sophisticated optical front-ends comprising separate illumination and detection paths, we propose to use axicon-like elements in a low-Fresnel-number regime, where they generate energy-efficient Bessel-like beams with a small number of sidelobes. This results in sensitivity penalties that remain well below 20 dB for practically useful DOF gains of up to 10x. Using scalar diffraction theory we show that the DOF/sensitivity trade-off of Bessel-like beams is determined only by their Fresnel number, and that their energy efficiency increases dramatically for low Fresnel numbers. Using a reconfigurable OCT sample arm incorporating a spatial light modulator we have generated a range of Bessel-like beams with different Fresnel numbers and benchmarked their DOF/sensitivity tradeoff using a scattering phantom and a biological sample. The experimental results are in good agreement with theory and yield important insights for the optical design of bulk-optic as well as miniaturized OCT systems that employ refractive or diffractive axicon-like structures to enhance the DOF.

8934-7, Session 2

Intravascular optical coherence tomography imaging at 3200 frames per second

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In conventional intravascular OCT system, the images are under sampled in the pullback direction because the sampling interval of 250 ?m is much larger than the transverse resolution, which is approximately 30 ?m. In clinical situations, the cardiac motion during acquisition will cause inaccuracy in frame spacing and possibly frame order, due to motion of the catheter along the vessel. We propose to solve these issues by further increasing the speed of intravascular OCT.

We demonstrate intravascular OCT imaging at 3200 frames per second (192,000 rpm scanning). This was achieved by using a custom-built catheter in which the circumferential scanning was actuated by a 1.0 mm diameter synchronous motor. The OCT system, with an imaging depth of 3.7 mm (in air), is based on a Fourier domain mode locked laser operating at an A-line rate of 1.6 MHz. The diameter of the catheter is 1.1 mm at the tip. Ex vivo images of human coronary artery (78.4 mm length) were acquired at a pullback speed of 100??mm/s. True 3D volumetric imaging of the entire artery, with dense and isotropic sampling in all dimensions, was performed in <1 second acquisition time.

The ultrafast intravascular OCT scanner we demonstrate in this study allows the acquisition of a fully sampled 3D data set of a coronary artery in less than a second. The shorter procedure will eliminate cardiac motion artifacts and further reduce the amount of flush media needed for imaging. It also significantly improves the longitudinal rendering of the pullback.

8934-8, Session 2

Tethered Capsule OCT Endomicroscopy

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Endoscopic examination of the upper gastrointestinal tract is costly and typically requires that the patient be sedated. Furthermore, video endoscopy only provides macroscopic information, thus biopsies must be excised in order to obtain a microscopic tissue diagnosis. In order to overcome limitations of endoscopy we have integrated the microscopic imaging capabilities of optical coherence tomography (OCT) with a tethered capsule that can be swallowed.

We have developed two different tethered capsule OCT endomicroscopy devices; the first generation had a diameter of 12.8 mm and a length of 25 mm and the second generation has a diameter of 11 mm. The second-generation device has a more robust tether that is designed to allow it to withstand multiple sterilization cycles. We have also fabricated a more advanced tethered capsule endomicroscopy device that contains OCT scanning optics at the base of the capsule and a white light video camera for navigation through other portions of the gastrointestinal tract.

We have tested OCT capsule endomicroscopy devices in 35 procedures conducted in healthy volunteers and patients with diagnosis of Barrett's esophagus. In 17 cases performed with the second-generation capsule, we observed similar image quality to that of the first-generation capsule, while at the same time improving patient acceptance and increasing the number of reuses from 2 to 5. The clinical prototype of the video-enabled OCT tethered capsule device was successfully tested in swine in vivo, demonstrating high quality OCT and video imaging of the esophagus, stomach, and small intestine.

Our initial experience has shown that tethered capsule endomicroscopy

provides 3-dimensional microscopic images of the entire esophageal wall in a rapid and well-tolerated manner. Inclusion of a video camera in the capsule has the potential to open up additional medical applications by facilitating capsule navigation in the stomach and small intestine.

8934-9, Session 2

Diffractive endoscope for ultrahigh-resolution SD-OCT imaging at 800 nm

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We present a novel OCT endoscope for ultrahigh-resolution 3D imaging at 800nm. A diffractive lens was developed for correcting chromatic aberration in the endoscope. With a broadband 800nm light source of a full wavelength range ~240nm and a miniature compound lens, the endoscope achieved a resolution of 3.75μm (axial) x 6.15μm (lateral) in air. Real time 3D OCT imaging was performed on ex vivo guinea pig esophagus and rat trachea. The results were compared with those acquired by an otherwise identical endoscope but without a diffractive lens, demonstrating the potential of the diffractive endoscope for ultrahigh-resolution intraluminal imaging.

8934-10, Session 2

Towards intravascular two- and three-dimensional flow measurements based on speckle decorrelation using optical frequency-domain imaging

Néstor Uribe-Patarroyo, Martin L. Villiger, Brett E. Bouma, Wellman Ctr. for Photomedicine (United States)

Flow measurements are critical to evaluate the clinical significance of a stenosis, and they drive the decision-making process regarding intervention. Two- and three-dimensional flow measurements could provide additional insight useful for this process, but more importantly could enhance our understanding of the pathogenesis of lesions and the role of endothelial shear stress under non-laminar and turbulent flow conditions. Two-dimensional intravascular optical frequency-domain imaging (IV-OFDI) flow measurements based on the Doppler effect require stringent phase stability in the acquisition system between different A-lines, and usually need a posteriori phase compensation techniques for the correction of the phase information in the tomograms. As the use of a rotating catheter introduces further complications in the phase compensation algorithms, the development of a flow measurement technique that relies only on the intensity information is highly desirable. Previous work has demonstrated flow measurements in bench-top scanning systems using measurements of speckle cross-correlation between A-lines, but this approach generally suffers from noise, caused by the statistical fluctuations of speckle. In this work, we present flow measurements based on speckle decorrelation in a catheter-based intravascular OFDI system, in which a specialized autocorrelation function optimized for the determination of the speckle size is used, leading to more accurate flow profiles than those previously reported in speckle decorrelation flow measurements. We demonstrate two- and three-dimensional flow profile reconstruction at 10 fps in a flow phantom setup, using intralipid at 0.5% volume concentration and parabolic laminar flow raging from 0 mL / min to 80 mL / min in 3.2 mm-diameter tubing.

8934-11, Session 2

In vivo imaging of the human upper airway using long range optical coherence tomography

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Obstruction in the upper airway can often cause reductions in breathing or gas exchange efficiency and lead to rest disorders such as sleep apnea. Imaging diagnosis of the obstruction region has been accomplished using imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI). However CT requires the use of ionizing radiation, and MRI typically requires sedation of the patient to prevent motion artifacts. Long-range optical coherence tomography (OCT) has the potential to provide high-speed three-dimensional tomographic images with high resolution and without the use of ionizing radiation. We present work on the advancements of our long range OCT system. Imaging is achieved thru the use of 3 types of endoscopic probes, 2 proximal rotation designs with outer diameters of 1.4 mm and 0.7 mm and 1 distal rotation design featuring a new short length micromotor. Imaging from the bottom of the larynx to the end of the nasal cavity is completed within 40 s.

8934-12, Session 2

Compensating for polarization mode variation in catheter based OFDI

David C. Adams, Alyssa J. Miller, Melissa J. Suter, Massachusetts General Hospital (United States)

Polarization sensitive optical coherence tomography (PS-OCT) has proven to be a useful tool for imaging collagen rich tissue, due to the form birefringence exhibited by said tissue. Extracting the magnitude of tissue birefringence is sufficient in a number of applications, but the ability to also obtain information about the orientation of birefringent tissue could be extremely beneficial. However, technical limitations have presented a significant hurdle in obtaining such information in fiber based imaging systems, both in bench top and catheter configurations. With an imaging catheter the difficulty is especially severe, since catheter rotation causes the measured optic axis to vary continuously over a single image frame. In this work we present a technique for deriving information about the orientation of birefringent tissue in data collected with an imaging catheter. This technique is an algorithm that can be applied during data processing, and which does not require any additional equipment or system modification. The algorithm functions by calculating the optic axis of the catheter sheath at multiple points along the frame and using the results to assemble a rotation matrix that, when applied to the entire depth profile, will partially correct for the spurious variations measured within the tissue. Since the sheath itself is nearly transparent, the axis is calculated from light reflected at the sheath boundaries. The success of the algorithm is validated with results obtained from ex vivo imaging of swine airway.

8934-13, Session 3

Coherence Revival Multiplexed, Dual Spot, Buffered Swept Source Optical Coherence Tomography: 400 kHz with a 100 kHz Commercial Source

Derek Nankivil, Al-Hafeez Dhalla, Kevin Shia, Sina Farsiu, Joseph A. Izatt, Duke Univ. (United States)

To increase the speed of swept source optical coherence tomography (SSOCT) systems, engineers have made lasers that sweep more rapidly, increased the effective duty-cycle of the laser through sweep buffering, and multiplexed the system by illuminating and collecting from multiple sample spots simultaneously. Sweep buffering is a technique for multiplying the sweep rate of swept-wavelength lasers. It is typically achieved using spools of optical fiber to create delayed copies of the sweep and fused fiber couplers to interlace the delayed sweeps. Also, multi-spot SSOCT systems previously demonstrated require a separate interferometer, reference arm, and receiver for each channel. In this paper, we describe an efficient method to quadruple the imaging speed using sweep buffering and a dual spot sample arm multiplexed using coherence revival. Sweep buffering was achieved using a spool of optical fiber and a fast optical switch. The dual spot sample arm was created using a polarizing beam splitter and fold mirror assembly. Coherence revival allowed for separate locations within the sample to be simultaneously imaged and frequency encoded. The coherence revival based technique for dual beam SSOCT is the first demonstration of beam-multiplexing that preserves full system efficiently without requiring additional reference arms, interferometers and receivers. Together, these methods can be used to efficiently quadruple the imaging speed of any SSOCT system employing a low duty cycle laser that exhibits coherence revival.

8934-14, Session 3

On-chip spectrometer for low-cost optical coherence tomography

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Spectral domain optical coherence tomography (SD-OCT) has become a viable modality for acquiring volumetric images of biological tissue and of non-biological materials. There has been some commercial success for SD-OCT, particularly in ophthalmic imaging, but these systems are often large, bulky, expensive, and delicate. Expansion of OCT into new markets, such as non-destructive testing (NDT) or point-of-care medical imaging, will require systems that scale better to these operating environments and market volumes.

An optical platform that can address several of the current challenges facing the widespread adoption of OCT is integrated optics, where waveguide-based optical systems are patterned on a silicon wafer. Devices microfabricated in this way have several important qualities including: robustness, small form factor, polarization-sensitivity, and low costs. These advantages enable the design of complex optical systems within a form factor that can be significantly smaller and less expensive than a free-space counterpart.

Towards the goal of a completely on-chip OCT system, Tornado Spectral Systems has developed a new spectrometer called OCTANE, the Optical Coherence Tomography Advanced Nanophotonic Engine, consisting of chip-based spectrometers for SD-OCT systems. Our commercial prototypes include a NIR system centered at 860 nm, a bandpass of 70 nm, and 2048 output channels that can record TE and TM polarizations independently at an 80 kHz line scan rate. OCT images were acquired and a 9.6 µm axial resolution and 2.7 mm imaging depth were demonstrated. Intended to support low-cost, high-volume applications, these spectrometers are well-suited to SD-OCT for both biological and industrial non-destructive testing applications.

8934-15, Session 3

Space-division multiplexing optical coherence tomography

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High speed, high resolution and high sensitivity are desirable for optical coherence tomography (OCT). Here, we demonstrate a space-division multiplexing (SDM) technology that translates long coherence length of a commercially available wavelength tunable laser into high OCT imaging speed. We achieved an effective 800,000 A-scans/s imaging speed using a 100,000 Hz tunable vertical cavity surface-emitting laser (VCSEL). A sensitivity of 94.6 dB and a roll-off of < 2 dB over ~30 mm imaging depth were measured from a single channel in the prototype SDM-OCT system. An axial resolution of ~11 um in air (or ~8.3 um in tissue) was achieved throughout the entire depth range. An in vivo, 3D SDM-OCT volume of an entire Drosophila larva consisting of 400 x 605 A-scans was acquired in 0.37 seconds. Synchronized cross-sectional OCT imaging of three different segments of a beating Drosophila larva heart is demonstrated. The SDM technology provides a new orthogonal dimension for further speed improvement for OCT with favorable cost scaling. SDM-OCT also preserves image resolution and allows synchronized cross-sectional and three-dimensional (3D) imaging of biological samples, enabling new biomedical applications.

8934-16, Session 3

Spectrally multiplexed imaging for swept-source optical coherence tomography using tunable virtually imaged phase arrays

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High speed imaging in optical coherence tomography (OCT) enables better diagnosis by because it provides for sampling of larger fields of view with decreased the imaging time and fewer motion artifacts. Swept-source OCT has shown remarkable enhancements in imaging speed by developing light sources with higher sweep rates. Alternatively SS-OCT can further gain from parallelization via multiple beam illumination. However, such systems require multiple detectors and data-acquisition devices, which is complicated and expensive.

Here we introduce a novel parallel acquisition scheme for SS-OCT that deploys a low-cost, tunable virtually-imaged phased array (VIPA) based on a glass slide and D-shaped mirror. The VIPA divides the spectral range of the light source into unique wavelength sets in the sample arm that simultaneously encodes multiple A-scans from many lateral points within a single sweep, thereby accelerating imaging speed. Our method requires only a single optical detector and data acquisition device. The optical system is versatile and simple to design: the number of multiplexed A-scans is a tunable function of the size of the air gap. We demonstrate proof-of-principle experiments showing 8-fold enhancement in imaging speed over traditional OCT for B-scan and volumetric imaging.

8934-17, Session 3

Tracking both magnitude and direction of 2-D transverse motion with optical coherence tomography

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In optical coherence tomography (OCT), motion tracking is critical for the development of free-hand OCT system which requires correction of motion artifacts to improve image quality and flow measurement for micro-circulation study. Optical Doppler tomography (ODT) has been widely used in flow measurement and motion tracking, in axial direction. We have also developed methods for transverse flow measurement and transverse motion tracking based on speckle decorrelation analysis. However, our speckle decorrelation method only extracts the speed but not the direction of the motion. In this study, we propose a transverse motion tracking method which can determine both speed and direction of the motion. This method involves scanning the OCT beam circularly and processing the obtained three dimensional data with novel algorithms. A 2-D OCT dataset (Pseudo Bscan, abbreviated as pBscan) is obtained when the beam performs one circle of scanning. Sequentially acquired pBscans form a 3-D data set (Pseudo Cscan, abbreviated as pCscan). We further averaged pCscan in axial dimension to form a 2-D image that has striped patterns as feature related to motion. Due to the motion, different Ascans in pBscan sample the same point in the transverse plane; therefore the striped patterns are observed. The obliquity of the striped patterns depends on the magnitude of motion and the location of the striped patterns depends on the direction of motion. We were able to extract parameters that are linearly related to the magnitude and the direction of motion with novel image analyzing methods.

8934-18, Session 3

Ultrahigh-resolution Spectral-domain OCT for micro-vascular imaging

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An ultrahigh-resolution spectral-domain optical coherence tomography (SD-OCT) system for visualization of microstructures including microvessels in vivo has been designed and implemented. With the use of a broadband superluminescent diode source (~240nm full width) we are able to obtain an axial resolution of 3.5μm, and through the use of a microscope objective, we have obtained a lateral resolution of 3.1μm confirmed by imaging of a standard USAF resolution chart. The performance of this system is demonstrated through in vivo and ex vivo imaging of nude mouse ear and in vivo cerebrovascular imaging of mouse with preliminary results from middle cerebral artery occlusion model.

8934-19, Session 3

Off-axis full-field swept-source OCT of ocular tissue

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We present an off-axis full-field swept-source OCT system for imaging different ocular tissues. The off-axis illumination introduces an angle between sample and reference light. This causes a shift of the signal term in Fourier space. By separation of the signal term from the DC and autocorrelated terms, as well as the complex conjugated term, coherence artifacts can be reduced significantly and full-range imaging becomes possible. However, this comes at the cost of lateral oversampling and thus a reduced field of view and/or lateral resolution. Furthermore higher numerical effort is needed for reconstruction.

Data of the anterior and posterior segment taken with this technique show less coherence noise and a by more than 10 dB increased signal-to-noise ratio compared to standard full-field swept-source OCT.

8934-20, Session 3

Frequency multiplexed long range swept source optical coherence tomography

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We present a novel swept source optical coherence tomography configuration, equipped with acousto-optic deflectors that can be used to simultaneously acquire multiple B-scans OCT images originating from different depths. The sensitivity range of the configuration is evaluated while acquiring five simultaneous B-scans. Then the configuration is employed to demonstrate long range B-scan imaging by combining two simultaneous B-scans from a mouse head sample.

8934-21, Session 4

Imaging of human cone and rod photoreceptors in vivo using SLO/OCT with adaptive optics

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In this study we investigate the 3D structure of human cone and rod photoreceptors of healthy volunteers. We use a transverse scanning optical coherence tomography (OCT) / scanning laser ophthalmoscope (SLO) instrument that is equipped with adaptive optics and active axial eye tracking. The instrument provides high lateral and axial resolution. The active eye tracking yields 3D information of the photoreceptor structure with negligible eye motion artifacts. Foveal cones and individual rod photoreceptors are visualized.

8934-22, Session 4

Hemodynamic imaging of the human retina using ultrahigh speed swept source optical coherence tomography

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One of the most metabolically active tissues in the body, the retina requires sufficient oxygen and nutrients from retinal and choroidal blood flow for normal retinal function. Therefore, assessing and visualizing ocular hemodynamics in vivo is important for understanding pathophysiology of retinal diseases such as age-related macular degeneration, diabetic retinopathy, and glaucoma, major causes of vision loss and impairment. In this study, we investigated Doppler OCT and OCT angiography and the effect of visual flicker stimulus on ocular hemodynamics. An ultrahigh speed swept source OCT prototype using a 400kHz A-scan rate VCSEL swept light source at 1060nm wavelengths

was developed. Pulsatile total retinal arterial blood flow was measured with an en face Doppler OCT technique, and OCT angiograms were generated using OCT speckle decorrelation angiography. The effect of visual flicker stimulus due to neurovascular coupling was measured quantitatively at the levels of total retinal blood flow and the capillary network. A transient increase in blood flow in response to flicker stimulus could be observed in both total retinal blood flow and retinal capillaries. These results demonstrate the potential of ultrahigh speed swept source OCT for hemodynamic imaging in the human retina. As a non-invasive in vivo imaging technique, these methods will enable longitudinal as well as cross-sectional studies in patients, and may be useful for the investigation of ocular diseases such as diabetic retinopathy and glaucoma.

8934-23, Session 4

Real-Time Pupil Tracking for Motion Corrected Anterior Segment Optical Coherence Tomography

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Conventional optical coherence tomography (OCT) systems do not capture volumes instantaneously and are therefore subject to artifacts due to patient motion. While a subject's voluntary motion may be mitigated with a fixation target, involuntary motion such as micro saccades, drifts, or tremors may still corrupt an OCT volume and associated en face summed volume projections (SVPs). In this work, a simple and robust method for real-time lateral movement compensation was employed using pupil tracking, a well-established video oculography technique. We integrated pupil tracking hardware and software into a custom, anterior-segment swept-source OCT system to demonstrate real-time movement compensation. To acquire images of the ocular pupil, we utilized a separate camera and near-infrared LED illumination source. Binary morphology image processing techniques were used to locate and track the pupil center and produce a voltage offset for the scanning mirrors to realign the OCT scans in real-time. OCT volumetric data were acquired with and without pupil tracking. The addition of real-time pupil tracking mitigated lateral motion artifacts within OCT volumes. These results are also evident within summed-volume projection (SVP) images, in which image distortion is clearly minimized with tracking.

8934-24, Session 4

Ultra-wide-field MHz-OCT-imaging with 85° viewing angle

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Ophthalmic imaging over an ultrawide field of view can improve the quality of clinical diagnosis. While other ultrawide-field retinal imaging modalities like scanning laser ophthalmoscopes can achieve more than 100° field of view, current commercial OCT systems cover only up to ~45°. Here we demonstrate an OCT system optimized for extremely wide scan angles. We present for the first time single shot densely sampled 3D OCT imaging over ~85° field of view (170° center angle) of a non-mydriatic eye. The angle is limited by clipping at the subject's iris. An entire data set consisting of 2088x2088 A-scans is acquired in ~3.5s at a 1.68MHz line rate. The system uses an FDML laser operating at a center wavelength of 1060nm with a wavelength sweep range of 60nm. Both, 6dB sensitivity roll off and OCT imaging range are ~3.5mm. The three major challenges associated with such a wide scan angle will be quantified, characterized and discussed: (a) The distortion of the beam profile for increasing scan angles, (b) the transverse beam walk off from the pivot point and (c) the increasing optical path length difference towards the periphery. Therefore, wide field OCT imaging requires sufficient OCT depth range and a careful optical design, which will be presented in detail.

8934-25, Session 4

Application of adaptive lens in sensorless AO-OCT for in vivo mouse retinal imaging

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We demonstrate Adaptive Optics - Optical Coherence Tomography (AO-OCT) with modal sensorless Adaptive Optics correction with the use of novel Adaptive Lens (AL) applied for in-vivo imaging of mouse retinas. The AL can generate low order aberrations: defocus, astigmatism, coma and spherical aberration that were used in an adaptive search algorithm. Accelerated processing of the OCT data with a Graphic Processing Unit (GPU) permitted real time extraction of image projection total intensity for arbitrarily selected retinal depth plane to be optimized. Wavefront sensorless control is a viable option for imaging biological structures for which AO-OCT cannot establish a reliable wavefront that could be corrected by wavefront corrector. Image quality improvements offered by the adaptive lens with sensorless AO-OCT was evaluated on in vitro samples followed by mouse retina data acquired in vivo.

8934-26, Session 4

Dual-beam Doppler OCT for complete angle independent flow measurement

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In vascular plexuses perpendicular to the optical axis, traditional Doppler OCT is highly sensitive to the Doppler angle, limiting its reproducibility and accuracy in clinical practice. A more stable approach is the dual-beam bidirectional technique that probes the sample from two distinct illumination directions allowing reconstruction of the true flow velocity independently of the Doppler angle. Later the blood flow can be calculated by knowledge of the vessel diameter. However the absolute velocity determination requires the evaluation of the flow angle in the en face plane, thereby shifting the angular dependence to another plane. We suggest calculating the flow directly from a Doppler cross section perpendicular to the illumination plane subtended by both illumination directions. Since both the vessel cross section and the measured dual-channel velocity scale with the vessel angle but inversely, the angular dependence cancels each other in the flow calculation. The principle is implemented with Swept Source OCT at 1060nm with 100,000 A-Scans/s. We demonstrate, with in vitro measurements of a perfused capillary that this method employing two beams only is completely independent of any vessel orientation in a broad angular range. We confirm the accuracy of the method in vivo by assessing a selected human retinal artery at different positions.

8934-27, Session 4

Dual beam Doppler FD-OCT system with integrated Dynamic Vessel Analyzer and rotatable beams to measure total retinal blood flow

Veronika Doblhoff-Dier, René M. Werkmeister, Medizinische Univ.

Wien (Austria); Martin Gröschl, Technische Univ. Wien (Austria); Leopold Schmetterer, Medizinische Univ. Wien (Austria)

We present a method to measure the total retinal blood flow in arteries and veins based on dual beam Fourier-domain Doppler optical coherence tomography (OCT) in combination with a commercially available retinal vessel analyzer (RVA) by Imedos GmbH (Jena, Germany). Incorporating an RVA into the system not only gives a live image of the fundus, it also allows determining the vessels' diameter precisely during the OCT measurement. Consequently, blood flow can be calculated by concomitant assessment of vessel diameter and blood velocity. While dual beam systems with fixed detection plane allow only vessels with certain orientations to be measure, the detection plane of our system can be rotated by 90°. Thus, the blood's velocity can be measured in all the vessels around the optic nerve head (ONH).

The OCT measurements were performed in a square scanning pattern around the ONH, in a distance of about one ONH-diameter from the margin of the ONH. In order to average data from several pulse periods and obtain the mean velocity, the measurement time was about five seconds. Additionally, the fundus region was recorded with the RVA. This allows the vessel diameters to be measured concomitantly with the OCT data.

The results are in the same range as previously published results of total blood flow measurements. Additionally, the high degree of conformity between the measured venous and arterial flow corroborated the system's validity. Hence, the system offers a high potential for examining retinal blood flow in patients with ocular disease.

8934-28, Session 4

Absolute retinal blood flow measurement with a dual-beam Doppler optical coherence tomography

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PURPOSE. To test the capability of a novel dual-beam Doppler optical coherence tomography (OCT) technique for simultaneous in vivo measurement of the Doppler angle and, thus, the absolute retinal blood velocity and the retinal flow rate, without the influence of motion artifacts.

METHODS. A novel dual-beam Doppler spectral domain OCT (SD-OCT) was developed. The two probing beams are separated with a controllable distance along an arbitrary direction, both of which are controlled by two independent 2D optical scanners. Two sets of optical Doppler tomography (ODT) images are acquired simultaneously. The Doppler angle of each blood vessel segment was calculated from the relative coordinates of the centers of the blood vessel in the two corresponding ODT images. The absolute blood flow velocity and the volumetric blood flow rate can then be calculated. To measure the total retinal blood flow, we used a circular scan pattern centered at the optic disc to obtain two sets of concentric OCT/ODT images simultaneously.

RESULTS. We imaged two normal human subjects at ages of 48 and 34. The total retinal blood flows rates of the two human subjects were calculated to be 47.01 ?l/min (older subject) and 51.37 ?l/min (younger subject), respectively. Results showed that the performance of this imaging system is immune to eye movement since the two sets of ODT images were acquired simultaneously.

CONCLUSIONS. The dual-beam OCT/ODT system is successful in measuring the absolute retinal blood velocity and the volumetric flow rate. The advantage of the technique is that the two sets of ODT images used for the calculation are acquired simultaneously, which eliminates the influence of eye motion and ensures the accuracy of the calculated hemodynamic parameters.

8934-85, Session PMon

A novel dispersion-based wavelength-swept and wavelength-stepped laser source for optical coherence tomography

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Optical-domain subsampling enables Fourier-domain optical coherence tomography (OCT) imaging at high-speeds and extended depth ranges while limiting the required acquisition bandwidth. Optical subsampling requires rapid wavelength stepped rather than wavelength swept sources. This preliminary study proposes and presents a novel wavelength-stepped laser source. The design is based on optical dispersion and includes a Fabry Perot etalon with a free space spectral range of 200 GHz. The resulting source provides a sweep rate of ~9 MHz over 48 nm range at a center wavelength of 1550 nm was obtained with 200 GHz steps.

8934-86, Session PMon

Towards a comprehensive eye model for zebrafish retinal imaging using full-range spectral domain optical coherence tomography

Maria Gärtner, Universitätsklinikum Carl Gustav Carus Dresden (Germany); Anke Weber, DFG-Ctr. for Regenerative Therapies Dresden (Germany); Peter Cimalla, Felix Köttig, Universitätsklinikum Carl Gustav Carus Dresden (Germany); Michael Brand, DFG-Ctr. for Regenerative Therapies Dresden (Germany); Edmund Koch, Universitätsklinikum Carl Gustav Carus Dresden (Germany)

In regenerative medicine, the zebrafish is a prominent animal model for studying degeneration and regeneration processes, e.g. of photoreceptor cells in the retina. By means of optical coherence tomography (OCT), the morphology of an individual fish retina can be followed in vivo over weeks reducing the variability of the results. To allow improvement of zebrafish retinal OCT imaging by suitable optics, we developed a zebrafish eye model using in vivo obtained geometrical data of the eye revealed by dispersion encoded full-range OCT together with a dispersion comprising gradient index (GRIN) lens model on the basis of refractive index data found in the literature. Using non-sequential ray tracing, the focal length of the spherical GRIN lens (diameter of 0.96 mm) was determined to be 1.22 mm at 800 nm wavelength resulting in a Matheissen's ratio (ratio between focal length and radius of lens) of 2.54, which fits well into the range of 2.4 and 2.82 found for various fish lenses. Additionally, a constant refractive index of 1.64 at 800 nm could be retrieved for the lens to yield the same focal position as found for the GRIN condition. With the aid of the zebrafish eye model, the optics of the OCT scanner head were adjusted to provide high-resolution retinal images with a field of view of 30° ? 30°. The introduced model therefore provides the basis for optical improvement of retinal imaging with OCT and can further be used to study the image formation within the zebrafish eye.

8934-87, Session PMon

Polarization-sensitive OFDI with polarization-multiplexed wavelength-swept light source

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Optical frequency domain imaging (OFDI) is one of the Fourier

approaches and is conceptually a 2- or 3-dimensional extension of optical frequency domain reflectometry (OFDR), which uses a wavelength-swept light source and interferometer for heterodyning. Recent advancement of imaging speed in OFDI utilizing orders of magnitude higher sensitivity and image acquisition speed opens the possibility of high-speed biological imaging of large tissue volumes. Polarization-sensitive OFDI (PS-OFDI) provides high-speed visualization of a cross-sectional birefringence map of a sample, such as the birefringence axes, the magnitude of birefringence, and polarization dependent scattering and attenuation coefficients, information that is complementary to standard intensity-based imaging. For polarization sensitive imaging in fiber-based OCT system, illuminating the sample with two polarization states that are perpendicular to each other on the Poincare sphere is required. Alternating polarization state of the incident light at the sample on successive A-lines by using a polarization modulator is the most widely used approach. Simultaneous illumination of the two frequency-encoded perpendicular polarizations in the sample arm to distinguish these polarization states on detection is another successful approach for PS-OFDI imaging. In this presentation, we demonstrate a PS-OFDI scheme that utilizes a polarization-multiplexed wavelength-swept light source. A novel wavelength-swept light source that alternates the polarization states of the laser output between adjacent A-lines without using a polarization modulator enables simple and robust PS-OFDI imaging.

8934-88, Session PMon

Maximum likelihood estimation of blood velocity using Doppler optical coherence tomography

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A recent trend in hardware advances in optical coherence tomography (OCT) has been the move towards higher A-scan rates. However, the estimation of axial blood flow velocities is limited by the presence and type of noise, as well as the estimation method. Higher acquisition rates alone do not enable the accurate quantification of axial blood velocity. Here we derive a maximum likelihood estimator (MLE) for Doppler frequency estimation that takes into account spectral broadening due to decorrelation. For in-vivo OCT measurements of blood flow, decorrelation noise affects Doppler frequency estimation by broadening the signal spectrum. We compare this estimator with existing techniques using flow phantom data. Both theory and experiment show that this estimator is effective, and is robust against inaccuracies in the estimate for the coherence time of the signal. We find that maximum likelihood estimation can be useful for estimating slow axial flow and near transverse flow. For estimating very low velocities using short acquisition times, the additive white Gaussian noise (AWGN) MLE has the best performance. For faster flows with more decorrelation, the decorrelation MLE performs the best. In addition, we outline the mathematical optimizations we have made to reduce the computational complexity of the decorrelation MLE scheme.

8934-89, Session PMon

Rotational Imaging OCT for Full-Body Embryonic Imaging

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Optical coherence tomography (OCT) has proved to be an effective tool to study the development of mammalian embryos due to its high resolution and contrast. However, light attenuation in biological tissues will place some practical limitations on the imaging depth of OCT due to strong light attenuation at later developmental stages (e.g. E9.5 and beyond). Here we propose a new method, named rotational imaging OCT (riOCT) that can improve the imaging depth and provide whole embryonic imaging. The experimental setup comprises the swept source OCT system and the square glass tube mounted on a rotational stage. The E9.5 mouse embryos are dissected and immobilized in the glass tube using 10% gelatin. 3D structural imaging is performed at four different angles with the interval of 90 degrees. The OCT image records the optical distances of different components such as glass, gelatin and tissue. The position of rotation center is determined by the track of the glass tube center at different angles. The final image is acquired by rotating the images at different angles according to the rotation center. The results demonstrate that rotational imaging OCT improves the visualization of whole mouse embryos.

8934-90, Session PMon

Variation in Cross-Correlation as a discriminator for microvessel imaging using clinical intracoronary Optical Coherence Tomography systems

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Cross-correlation of Intracoronary Optical Coherence Tomography (OCT) images is affected by image distortion due to the non-uniform rotational velocity of the imaging catheter. It results in non-representative cross-correlation maps such that for a static scan, the coefficients fluctuate from high to low correlation values. The variation in cross-correlation at flow locations is muted, in comparison to stationary regions. In the present study, the variation of correlation values and its standard deviation (SD) is used to suppress the distortion related noise effects and to extract flow maps from static scan images. The standard deviation of the cross-correlation variation can distinguish flow locations from the surrounding tissue region. The advantage of this technique is its ability to identify slow flow, even Brownian flow, in the presence of motion artifacts. The SD mask used for generating flow maps, is optimized using tissue mimicking phantoms. Finally, the ability of this technique to suppress noise and capture flow maps is demonstrated by imaging microflow through excised porcine coronary artery wall and nailfold capillary imaging.

8934-91, Session PMon

Comparison of sampling and reconstruction strategies for Fourier Domain Optical Coherence Tomography

Evgeniy Lebed, Marinko V. Sarunic, Mirza Faisal Beg, Simon Fraser Univ. (Canada)

In this work we explore different sampling and reconstruction techniques for volumetric Spectral Domain Optical Coherence Tomography (SDOCT). Even though SDOCT is an established imaging modality in diagnostic ophthalmology, it still suffers from a numbers of serious drawbacks, including long acquisition times. In recent years there has been much interest in reducing the acquisition time by only scanning a certain subset of the target followed by image interpolation via sparsity-promoting optimization. Different sampling strategies that have recently been proposed include: 1) uniform raster – where a portion of B-scan is not collected at uniformly spaced intervals, 2) random raster – where a portion of B-scans is not collected at randomly spaced intervals and 3) random radial – where B scans are collected as radial spokes with a

random angular increment. In this work we investigate the performance of two sparsity promoting based interpolation methods, iterative soft thresholding (IST) and fast iterative shrinkage-thresholding algorithm (FISTA) on a set of ophthalmic SDOCT images acquired by the above-mentioned sampling strategies. The interpolation performance is evaluated in terms of quantitative image quality metrics (signal to noise ratio and mean squared error) as well as the changes in the reconstructed volumes' clinically relevant morphometric measurements (retinal nerve fiber layer thickness and total retinal thickness).

8934-92, Session PMon

Swept-source common-path optical coherence tomography with a MEMS endoscopic imaging probe

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A MEMS-based common-path endoscopic imaging probe for 3D swept source optical coherence tomography (SSOCT) has been developed. The common path is achieved by setting the reference plane at the rear surface of the GRIN lens inside the probe. MEMS devices have the advantages of low cost, small size and fast speed, which are suitable for miniaturizing endoscopic probes. The aperture size of the two-axis MEMS mirror employed in this endoscopic probe is 1 mm by 1 mm and the footprint of the MEMS chip is 1.55 mm by 1.7 mm. The MEMS mirror achieves large two dimensional optical scan angles up to 34 degree at 4.0 V. The endoscopic probe using the MEMS mirror as the scan engine is only 4.0 mm in diameter. Additionally, a method has been developed, in which an optimum length of the GRIN lens is used to remove the artifacts of the SSOCT imaging generated from the multiple interfaces inside the endoscopic imaging probe. The MEMS based common-path probe demonstrates real time 3D OCT images of human finger with 10.6 ?m axial resolution, 17.5 ?m lateral resolution and 1.0 mm depth range at a frame rate of 50 frames per second.

8934-93, Session PMon

Real-time FD OCT flow contrast imaging in the retina using GPU accelerated processing

Jing Xu, Kevin Wong, Yifan Jian, Sherry X. Han, Marinko V. Sarunic, Simon Fraser Univ. (Canada)

We report on real time acquisition and display of flow contrast images acquired with FD OCT in mouse and human eyes in vivo. Motion contrast from blood flow was processed using the speckle variance OCT technique, which relies on the acquisition of multiple B-scan frames at the same location and tracking the change of the speckle pattern. Results from two different custom build OCT systems are present used in this report. Human data was acquired using a 100kHz line rate Swept Source OCT in the 1060nm wavelength range. Mouse data was acquired using a Spectral Domain OCT system with line rates up to 180kHz in the 800nm wavelength range. Real time processing and display was performed using a custom GPU platform, and included structural OCT data, en face flow contrast images, and en face speckle variance(sv) en face flow contrast images of the retinal vascular layers. The human data was processed using speckle variance techniques during the acquisition, and permitted a high quality image using the real time svOCT for image guidance.

8934-94, Session PMon

Assessment of the flow velocity of blood components in a microfluidics device using spectral and time domain optical coherence tomography

Danuta M. Bukowska, Maciej Szkulmowski, Szymon Tamborski, Maciej Wojtkowski, Nicolaus Copernicus Univ. (Poland)

Although Optical Coherence Tomography is mostly used for imaging of tissue morphology, it is also capable of visualizing functional process in biological tissues. In particular, there has been a considerable interest in ocular blood flow assessment. Changes of retinal blood circulation are considered to be a condition associated with a number of eye diseases such as age related macular degeneration, glaucoma and diabetic retinopathy. Therefore, blood flow detection and measurement techniques may play an important role in ophthalmic diagnostics.

Several OCT techniques for flow detection in the eye have been introduced up to date. The most widely used include the phase resolved methods and resonant Doppler methods. Our group proposed a joint Spectral and time domain OCT method which can detect the flow based on the Doppler frequency shift. However, even if the Doppler OCT techniques have already enabled imaging of biological flow in mid-size vessels, the reconstruction of velocity maps of the capillary network still provides a challenge. In the flow maps of capillary network randomly varying Doppler signal is observed.

Therefore, in this study, we have investigated the ability of DOCT technique to measure velocity profiles of whole blood, concentrates of red blood cells and white blood cells, in a range of microchannel configurations. This is the first step to an understanding of single cells' flow behavior in microcapillaries using DOCT.

8934-95, Session PMon

Swept source optical coherence tomography for soft contact lens measurements

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For the purpose of the project we designed and constructed a dedicated swept source OCT (ssOCT) system. A commercial swept source laser (1300 nm AXSUN Technologies Inc., Billerica, MA) was used as a light source. Laser operated at central wavelength of 1300 nm with repetition rate of 50 kHz (sweep period and so the exposure time was 12 µs). Laser bandwidth of 100 nm enabled imaging with axial resolution of 9 µm. With 200 MS/s acquisition board (Gage Applied Inc., Lockport, IL; Gage Compuscope 14200, 14 bit resolution) ~9 mm of imaging depth in air was obtained. Before measurement the holder has to be filled with a fluid (saline solution) to create convex meniscus. The contact lens (with lens edges directed upwards) placed on such prepared fluid surface is kept by the surface tension. Contact lens not only remains on the fluid surface but it is also automatically positioned at the center of the meniscus (and centered in respect to the wall of a cylindrical container). Furthermore, in the area of the contact lens additional concave meniscus is formed. To increase efficiency of automatic segmentation we apply inverse contrast imaging method. 2 drops of 30% intralipid dissolved in 15 ml of contact lens solution provides suitable conditions for such inverse imaging. Radial scanning protocols with different averaging methods were applied. Results of 3D imaging of soft contact lens are presented. Limitations and possibilities of the new apparatus are discussed.

8934-96, Session PMon

Temporal correlation of optical coherence tomography in-vivo images of rabbit airway for the diagnosis of edema

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Recently, the application of full-range optical coherence tomography (OCT) systems for human airway imaging has been developed, where a fiber-based OCT probe scans human airway rotating at high speed. Edema on human airway occasionally induces airway stenosis, the abnormal narrowing of the airway, which becomes a life-threatening and life-ending event for, especially, clinically-ill newborns. Therefore, early detection of edema is clinically very important. In order to probe the potential of diagnosing edema on the clinical translation of the full-range OCT system, we investigate temporal correlation of OCT airway images measured from live rabbits. Multiple rotations of an OCT probe at fixed locations of edema and normal regions on the rabbit airway acquire temporally correlated OCT images, where the edematous tissue is experimentally modeled by injecting saline underneath the epithelium layer. The calculated temporal cross correlation coefficient functions between OCT images measured on the normal airway show periodicity that correlates with a respiratory motion of the airway. However, the ones from OCT images of edematous regions show randomness without the periodic characteristic. These in-vivo experimental results of temporal correlation show the potential of a computer-based diagnosis of edema on human airway OCT imaging.

8934-97, Session PMon

Pulsed laser tissue marking for OFDI-guided biopsy

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Optical frequency domain imaging (OFDI) has enabled comprehensive volumetric microscopy of the upper gastrointestinal tract at fast acquisition rates in a catheter-based platform. Guiding tissue biopsy by OFDI could overcome the diagnostic uncertainty of conventional random four-quadrant sampling. To this end, OFDI-guided biopsy was recently demonstrated, employing an additional laser integrated into the OFDI probe to create spatial marks on the tissue surface. This paradigm enables spatial co-registration between OFDI and video endoscopy for longitudinal surveillance as well as the collection of tissue biopsy with precision. However, the current marking capability was substantially limited by the laser energy available at the tissue surface and inefficient heat deposition due to thermal diffusion under continuous-wave optical irradiation. Moreover, this required stationary irradiation with a complicated localization process that slows down the overall procedure.

Here, we demonstrate a high-energy pulsed Raman fiber laser (RFL) (>20 mJ/pulse) at 1.44-?m wavelength to greatly enhance the marking efficiency. The RFL can share a single optical probe with the OFDI system working in the 1.3-1.5 ?m range. Our RFL produces a high pulse energy exceeding by far the theoretical requirement for single-pulsed coagulation of tissues (here, ~1 mJ). We first characterized the RFL output in terms of spectra and Raman-converted energy. Then, we generated visible marks ex vivo on tissues, using a pulse width of 50~500 ?s that approaches the A-line acquisition time of OFDI.

8934-98, Session PMon

Speckle reduction for OCT images using wave atoms thresholding filtering

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Optical coherence tomography is an emerging noninvasive imaging technique which performs high-speech and cross-sectional tomography in vivo and/or in vitro imaging of tissue microstructure with micrometer scale resolution. However, OCT images suffer from a special kind of noise called “speckle” which significantly degrades the OCT imaging quality. Here, we present an effective shrinkage filter for speckle reduction in OCT images based on wave atoms transform. Wave atoms is a new multiscale geometric analysis tool which can offer a sparser expansion and a better representation for images containing oscillatory patterns and textures than other traditional transforms, such as wavelet and curvelet transforms. Moreover, due to lack of translation invariance of wave atoms transform, cycle spinning based technology is introduced to avoid visual artifacts, such as Gibbs-like phenomena, and to develop a translation invariant wave atoms denoising scheme. The speckle attenuation degree is controlled by a single parameter that determines the threshold in the wave atoms domain. The experiment results show that the proposed method can remove the speckle noise and obtain better improvement of OCT image quality both visually and in terms of signal to noise ratio (SNR), contrast-to-noise ratio (CNR), and average equivalent number of looks (ENL) compared to wavelet and curvelet thresholding techniques.

8934-99, Session PMon

Longitudinal optical coherence tomography attenuation mapping to study the effect of temperature and fixation in ex-vivo atherosclerotic tissue characterization

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Ischemic heart disease is the major cause of death around the world and rupture of vulnerable atherosclerotic plaques is the biggest contributor to the phenomenon. The tissue composition of the plaque is an important predictor of its vulnerability.

Optical coherence tomography (OCT) is widely used for intravascular imaging. Since different tissue types attenuate light to a different extent and therefore we aim to use the attenuation coefficient obtained from OCT backscattered signal to distinguish between tissue types. Imaging the local attenuation coefficient provides a way to assist interpretation of OCT images of coronary plaques. We study the relation between plaque type and tissue optics using ex-vivo models. Tissues are better preserved during the experiments when kept at low temperature and/or formalin fixed. In the current study, we aim to quantify the difference in optical parameters, if any, between in-vivo and ex-vivo conditions.

The ex-vivo model we used consists of a whole heart cadaveric specimen. OCT imaging of the coronary arteries was performed at different temperature conditions and pre and post fixation. The OCT data was analyzed using a mathematical model for OCT signals to retrieve the attenuation coefficient. To do the comparison between different conditions we have developed a display in which the attenuation data of the entire pullback, sampled between 100-200 microns from the lumen border, is depicted as a color coded longitudinal map. The map highlights various tissue types in the pullback and such maps from the different conditions are compared with the one from the body temperature without fixation as base condition i.e. close to in-vivo conditions. The map image is processed in blocks to obtain the local median values.

The proportionality of these values for different conditions with the base condition is calculated. The results show that the proportionality or the slope of the line through the origin fitted for the medians is close to unity for every condition. The results suggest that fixation and room temperature conditions in the ex-vivo OCT experiments do not induce a systematic error.

8934-100, Session PMon

Physical attributes and assembly of PEG-linked immuno-labeled gold nanoparticles for OCM image contrast in tissue engineering and developmental biology

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Excessive nonspecific binding often occurs when labeling cells with immuno-labeled gold nanoparticles (IgG-AuNPs). We have investigated the physical properties of IgG-AuNPs assembled with three different protocols in an attempt to understand and eliminate this non-specific binding. One of these protocols involves conjugating the secondary antibody AP124F via van der Waals (vdW) and/or electrostatic forces to the AuNPs, and the other two employ a PEG-linker, OPSS-PEG-NHS (OPN). In all three protocols we follow with PEG-SH to provide protection against aggregation in saline solution. OPN and PEG-SH chains of varying molecular weights were examined in different combinations to determine the optimally protective layer. The hydrodynamic radius and surface plasmon resonance (SPR) were monitored at each stage of assembly using a dynamic light scattering (DLS) instrument and spectrophotometer, respectively. SPR measurements indicate a different physical structure near the gold surface when the PEG-linker is bound to gold first and then bound to the antibody second (AP124F-[OPN-Au]) rather than vice versa ([AP124F-OPN]-Au). These observed structural differences may lead to differences in the amount of non-specific binding observed when immuno-labeling cells. SPR measurements also yielded a half-time of 27 minutes for the binding of the PEG-linker to the surface of the AuNPs and a half-time of 133 minutes for the hydrolysis of the NHS functional groups on the OPN molecule. These different reaction rates led us to add AP124F 40 minutes after the linker began binding to the AuNPs, so that the antibody can bind covalently to the correct end of the OPN linker.

8934-101, Session PMon

Robust, real-time, digital focusing for FD-OCM using ISAM on a GPU

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Frequency domain optical coherence tomography (FD-OCT) achieves high image acquisition speeds by probing all depths of a sample simultaneously. However, the tightly focused beam required for frequency domain optical coherence microscopy (FD-OCM) produces images with poor lateral resolution at depths away from the beam waist. The new technique of interferometric synthetic aperture microscopy (ISAM) can digitally focus these poorly resolved FD-OCM images, resulting in uniform lateral resolution throughout the sample volume equivalent to that in the plane of focus of the incident beam. While ISAM is computationally intensive, we demonstrate that an ISAM implementation using Nvidia's parallel compute unified device architecture (CUDA) can achieve real-time focusing using a mid-range Nvidia GPU. Time required for digital focusing scales linearly with image size, at a rate of about 10 nanoseconds per voxel. This makes possible real-time FD-OCM. For example, a 3-D image (512 x 512 x 64 voxels) with cross-section 1.2 mm x 1.2 mm and 200 micron depth requires 17 seconds to acquire with a 100 kHz A-scan rate, but only 180 ms to focus with ISAM. This example image was simulated with a numerical aperture (NA) of 0.07, so that the 200 micron depth represents four Rayleigh ranges. In addition, our simulations indicate that ISAM performs well with very noisy input data. Even with noise levels as high as 50%, ISAM produces focused images with signal-to-noise ratios of over 100. ISAM-focusing is both fast and robust.

8934-102, Session PMon

O-band (1310 nm) Vernier-tuned distributed Bragg reflector (VT-DBR) device characterization for OCT

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Vernier-tuned distributed Bragg reflector (VT-DBR) lasers in source swept OCT (SS-OCT) have previously been demonstrated at 1550 nm and 1600 nm. Many OCT applications prefer 1310 nm operation. This work describes the first demonstration of a VT-DBR operating at 1310 nm in the O-band, ideal for use in SS-OCT. This paper addresses the device characterization of such lasers, illustrating they are capable of fast amplitude and frequency sweeps necessary for SS-OCT applications. Frequency modulation of the Front Mirror, Back Mirror, and Phase section of the VT-DBR laser at 1310 nm indicates sufficient rates to support OCT repetition rates of greater than 200 KHz. Equivalent circuit models for each of the five ports are also created to determine their electrical parasitics. The RC limitations of bandwidth of several GHz far exceed any modulation limitations, indicating the devices are not RC limited. Narrow linewidths of the VT-DBR indicate coherence length of several centimeters are possible during fast wavelength sweeps.

8934-103, Session PMon

Ultrahigh-phase-stable swept source based on KTN electro-optic deflector towards Doppler OCT and polarization-sensitive OCT

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We have developed a wavelength-swept laser source with ultrahigh phase stability. KTa1-xNbxO3 (KTN) single crystal was employed as an electro-optic deflector for a high-speed wavelength sweep. A 200-kHz sweep rate was obtained with a 20 mW average output power and an 8 mm coherence length at the wavelength range over 100 nm. Fast response of KTN crystal realized ultrahigh phase stability in the 1.3 ?m wavelength range. The standard deviation of timing jitters between adjacent A-lines was confirmed to be less than 167 ps. The ultrahigh phase stability makes our swept source promising for Doppler OCT and polarization-sensitive OCT.

8934-104, Session PMon

Full-field en face correlation mapping optical coherence tomography

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We have developed a full-field correlation mapping optical coherence tomography (FF-OCT) system for the purpose of performing non-scanning en face flow imaging. The light source is centered at 840 nm with a bandwidth of 50 nm and microscope objectives with a numerical aperture of NA = 0.1 were incorporated giving a transverse resolution of 5 μm and an axial resolution of 8 μm in air, both of which were confirmed experimentally. A magnification of 5.65 was measured, resulting in a field of view of 1260 ? 945 μm covered by the 1920 ? 1440 pixels of the camera. Pairs of interferometric images are captured, separated by a phase difference of π and a two-step phase image reconstruction method is applied to reconstruct each en face image. The OCT frame rate is 10 Hz. A two dimensional cross-correlation technique is applied to pairs of consecutive en face images in order to distinguish dynamic from static light-scatterers. The feasibility of the method was tested by simulating blood flow by creating a phantom with 5% intralipid solution within a glass capillary tube and simulations of both static and moving scatterers were performed.

8934-105, Session PMon

Stability analysis of polarization-based demodulation interferometers

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In this work, we analyze and improve the stability of polarization-based quadrature demodulation circuits to improve their utility in high-speed OCT systems. Interferometers that provide complex conjugate demodulation without acousto-optic frequency shifting may become important in next generation FD-OCT systems. Polarization-based demodulation techniques were previously demonstrated for this purpose, but offered limited stability and complex calibration procedures. We investigate the stability of polarization-demodulation interferometers and demonstration that with thermal stabilization, electrical phase matching, and new calibration algorithms, the use of polarization-based demodulation can be simplified. We also demonstrate this demodulation strategy in the context of polarization-diverse detection.

8934-106, Session PMon

Angle polished single mode fiber probe with optimized reference for a common-path optical coherence tomography

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Common-path optical coherence tomography (CP OCT) based on a single mode fiber (SMF) probe has been shown to be highly effective in endoscopic imaging and surgical tool guidance due to its simple design, miniature probe dimension, disposability. In CP OCT, the reference signal is usually derived from the fiber probe tip. Fresnel reflection at the interface between the fiber and air can provide about 3.4% of the incident light as a reference signal. However, this reference level is too high to obtain optimized OCT images. With a larger reference power, the camera has to be operated with a shorter integration time to avoid sensor saturation at which the detector output signal does not increase with the increasing number of incident photons. Therefore, the OCT system detects less signal photons which results in smaller signal magnitude. This implies that higher reference power does not necessarily lead to higher image SNR. In this abstract, we study and propose a method for reference optimization in CP OCT through angle polishing a SMF probe. We studied the dependency of reference level on the SMF probe tip angle. To obtain a decent signal-to-noise ratio (SNR) and autocorrelation noise suppression, we chose to polish the probe tip to 2 degree which led to a more than 8dB improvement in SNR compared to a flat tip SMF probe. The optimized angled SMF resulted in a significantly improved quality of OCT image.

8934-107, Session PMon

Multiple reference optical coherence tomography (MRO):

a miniature low coherence interferometeric imaging platform

Hrebesh M. Subhash, Martin J. Leahy, National Univ. of Ireland, Galway (Ireland); Josh Hogan, Carol Wilson, Compact Imaging, Inc. (United States)

Multiple reference optical coherence tomography (MRO™) is a recently developed miniature time-domain low coherence interferometeric imaging platform, which promises to fit into robust, cost-effective design: virtually solid state, typical of handheld devices. The key element of MRO is a multiple references optical delay based on piezoceramic (PZT) actuator and a partial mirror, which causes light to be reflected multiple times between the partial mirror and the reference mirror. The resulting multiple reflection orders provide a systematic increase in the magnitude of each successive scan region and the corresponding increase in the beat frequency of the interference signals associated with the multiple orders. A zero-crossing linearization algorithm is adapted for correcting the nonlinearities associated with the PZT scan actuator. In order to extract each scan segments corresponding to various successive multiple scan orders, a frequency resolved algorithm is implemented. Then this information from all the scan regions is stitched together to form an image.

8934-108, Session PMon

Interferometric synthetic aperture microscopy with automated parameter evaluation and phase equalization preprocessing

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A method of OCT imaging with a resolution throughout the investigated volume equal to the resolution in the best-focused region is described. It is based on summation of three-dimensional scattered field distributions at the wavelengths determined by OCT source spectral decomposition. A method of finding parameters needed for algorithmic realization of the summation is also proposed. The proposed approaches are tested on several model media, including biological ones. As the proposed algorithm is phase sensitive, and phase stability is crucial, phase equalization preprocessing which allows compensating the phase error caused by object motion during scanning was proposed.

8934-109, Session PMon

Toward absorption contrast imaging of biological tissues in vivo by using photothermal optical coherence tomography

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Optical coherence tomography has been proven in the last two decades its clinical value by providing 3D non-invasive in vivo biopsy of the biological samples. In addition to structural information given by the

backscattered intensity, the optical absorption will also provide another powerful contrast. Optical absorbers in biological tissues exhibits important role such as hemoglobin and melanin. However, current methods of absorption contrast take long time and not suitable for in vivo imaging.

Toward in vivo absorption contrast imaging, we developed photothermal OCT system by combining swept-source OCT system and excitation laser.

A swept-source OCT system is used with a wavelength swept laser at 1310 nm with a scanning rate and range of 47 kHz and of 100 nm, respectively. Photocurrents from balanced photoreceivers are sampled by a high-speed digitizer by using k-clock from the source to sample optical spectrum in k-linear domain. The sensitivity of 107 dB for two polarization channels is achieved. At the sample arm, the OCT probe beam and an excitation laser are combined by a dielectric mirror. The fiber-coupled laser diode of 406 nm wavelength is used for excitation since the absorption of hemoglobin has peak around this wavelength.

In order to evaluate the ability of this system, phase stability of the system was measured. The standard deviation of the phase shift is measured as 0.0028 radians, where the signal-to-noise-limited value is approximately 0.001. Several issues for in vivo case, motion, blood flow, thermal damage, and etc. will be addressed here.

8934-110, Session PMon

Design of pupil filter for extended depth of focus and lateral superresolution in optical coherence tomography

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In this work we propose spatially modulating the pupil function of the focusing optics by a seven-zone binary pupil filter. To achieve superresolution along an extended DOF, many designs of pupil filters have been reported, including amplitude filters, pure phase filters, and complex filters. Amplitude filters are not energy efficient since a part of the pupil is obstructed. Recently, attention has been shifting to the design of phase only pupil filters due to their improved transmittance which make them more practical. Four-zone binary phase pupil filters were used for superresolution along extended depth of focus in a Swept Source Optical Coherence Microscopy system. A gain in DOF from 8.9-11.5 and a superresolution factor of 0.7 has been reported by such filters. However, higher sidelobes are always observed in phase only filters. Complex pupil filters were introduced to improve the superresolving power of an optical imaging system11 assuming a uniform monochromatic optical wave. In this summary, the performance of a seven zone pupil filter especially designed for OCT is demonstrated. A Gaussian beam profile and a broadband source spectrum are considered to simulate the light beam in the sample arm of a typical OCT system. An increased depth of focus by 14 times and an improved lateral resolution by a factor of 1.47, maintained constant throughout the DOF, can be achieved using this filter

8934-111, Session PMon

High-sensitive full-range optical vibrometry based on Fourier-domain optical coherence tomography

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Optical vibrometery based on low coherence Fourier-domain optical coherence tomography (FD-OCT) technique are capable for providing depth resolved information in comparison with conventional laser based vibrometery. Recently, there has been growing interest in developing coherence-domain vibrometry for various clinical and pre-clinical applications. However, a major drawback of the conventional vibrometer based on Fourier-domain low coherence interferometry is the complex-conjugate ambiguity. This is because in FD-OCT, the detected real valued spectral interferogram is Fourier transformed to localize the scatter within the sample. The Fourier transform of a real valued function is Hermitian, so the reconstructed image is symmetric with respect to the zero-phase delay of the interferometer, leading to ambiguity in interpretation of the resulted OCT images. In this paper, we introduce a full range optical coherence vibrometry to utilize the whole imaging range of the spectrometer. The mirror image elimination is based on the linear phase modulation of the interferometer's reference arm mirror and with an algorithm that exploits Hilbert transform to obtain full range complex imaging.

8934-112, Session PMon

A new algorithm for speckle reduction of optical coherence tomography images

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OCT is based on low-coherence interferometry, which uses the spatial and temporal coherence properties of optical waves backscattered from a tissue sample to form an image. Images obtained from OCT are confounded by speckle. In an optical imaging system, speckle imposes a grainy texture on images produced and reduces their signal-to-noise ratio (SNR) and their contrast to noise ratio (CNR). Speckle also decreases the spatial resolution of the image, concealing subtle differences inside microstructures within a tissue sample. Here we present a new algorithm based on an artificial neural network (ANN), for reducing speckle noise from optical coherence tomography (OCT) images. The noise is modeled for different parts of the image according to its scattering coefficient using Rayleigh distribution, each of which with a noise parameter, sigma, estimated by the ANN. The input to the ANN is a set of intensity and wavelet features computed from the image to be processed, and the output is an estimated sigma value. This is then used along a numerical method to solve the inverse Rayleigh function to reduce the noise in the image. The algorithm is tested successfully on OCT images of eye. It is demonstrated that the signal-to-noise ratio (SNR) and the contrast of the processed images are increased by the application of the ANN algorithm in comparison with the respective values of the original images.

8934-113, Session PMon

Imaging of Neuronal Tissue Using a Prism Adjunct

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We present the use of a prism as an imaging adjunct with a multimodal system of optical coherence tomography (OCT) and confocal microscopy operating at 1320nm and 970nm respectively. The confocal microscopy and OCT systems combine paths using a 1?m dichroic beamsplitter and focus upon the sample through a x20 magnification microscopic objective lens. A 0.5mm triangular glass prism was inserted into an intact ex-vivo formalin fixed murine brain. The prism was placed upon the underside of the brain so that the optical system is aimed towards the optic nerves and optic chiasm.

The area just in front of the prism was scanned, en-face, using both confocal and OCT imaging techniques. The sample was then translated so that the focal point was at the external face of the prism, then moved once more to visualise the exiting face of the prism, and finally once more to move the focal point just outside of the prism. The area was scanned with both confocal and OCT imaging.

A comparison between the two imaging collections show that although the penetration depth and a tiny section of tissue is compromised with the use of the prism, clear structures can still be seen; whilst also showing a different architecture that would not have normally been visualised without the presence of the prism. The combination of such imaging techniques could form a volume that has greater detail and interpretation of neuronal tissue than that of each individual technique.

8934-114, Session PMon

Wavelet decomposition for speckle reduction with feature preservation in optical coherence tomography

Evgenia Bousi, Panayiotis Ioannides, Costas Pitris, Univ. of Cyprus (Cyprus)

Optical Coherence Tomography (OCT) images exhibit the effects of speckle which can make image interpretation and quantitative measurements difficult. Many approaches have been developed to reduce this speckle including both hardware implementations and post-processing techniques. However, they either suffer from a loss in resolution and blurring of the image or an increase in complexity and reduction in speed of the system. Wavelet decomposition has been shown to effectively separate the resolvable features of an image from the speckle pattern. The two components can then be processed separately. The speckle pattern can be filtered and then recombined with the resolvable component to create an image with improved SNR and intact image details. The results of this algorithm are demonstrated on in vivo OCT images of skin taken with a swept-source based system. Such a technique, when applied, for example, to OCT images of disease, can be extremely useful in improving the clinical interpretation of the images as well as allowing more accurate quantitative measurements not affected by the presence of speckle.

8934-115, Session PMon

Towards using spectral domain optical coherence tomography for dental wear monitoring

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Aim and objectives. Pathologic tooth wear refers to the loss of dental hard tissues that is not caused by caries or macrotrauma.

In this paper we demonstrate that a fast spectral domain OCT imaging system has the potential to monitor the evolution of pathological dental wear.

Materials and methods: On 10 caries free teeth, four levels of artificially defects similar to those observed in the clinic were created. After every level of induced defect, OCT scanning was performed. B scans were acquired and 3D reconstructions were generated.

A swept source OCT instrument is used in this study. The swept source is has a central wavelength of 1050 nm and a sweeping rate of 100 kHz. A depth resolution determined by the swept source of 12 µm in air was experimentally measured.

Results. The loss of dental hard tissue is qualitatively observed on the B-scans as 2D images and 3D reconstructions (volumes). For quantitative evaluations of volumes, the Image J software was employed. The minimal volume, measured in air that our system could measure is 2352 µm3, being able to collect high resolution volumetric images in 2.5 s. By calculating the areas of the amount of lost tissue corresponding to each difference of B-scans, the final volumes of abfractions (0.1112 mm3 - 2.524 mm3) and incisal attritions (0.125 mm3 - 1.980 mm3) were obtained.

Conclusions: This spectral domain OCT method is a valuable tool for dynamic evaluation of the dental wear with remarkable potential for clinical use.

8934-116, Session PMon

Effect of contact lens on optical coherence tomography imaging of rodent retina

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PURPOSE: To evaluate the effect of powerless contact lens on improving the quality of optical coherence tomography imaging of rodent retina.

METHODS: A spectral-domain optical coherence tomography (SD-OCT) system was built for in vivo imaging of rodent retina. The calibrated depth resolution of the system was 3 µm in tissue. A commercial powerless contact lens for rat eye was tested in the experiments. For each rat eye, the retina was imaged in vivo sequentially first without wearing contact lens and then with wearing contact lens. The lateral resolution and signal-to-noise ratio of the OCT images with and without contact lens were compared to evaluate the improvement of image quality.

RESULTS: The fundus images generated from the measured 3D OCT datasets with contact lens showed sharper retinal blood vessels than those without contact lens. The contrast of the retinal blood vessels was also significantly enhanced in the OCT fundus images with contact lens. As high as 10 dB improvements in SNR was observed for OCT images with contact lens compared to the images of the same retinal area without contact lens.

CONCLUSIONS: We have demonstrated that the use of powerless contact lens on rat eye can significantly improve OCT image quality of rodent retina, which is a benefit in addition to preventing cataract formation. We believe the improvement in image quality is the result of partial compensation of the optical aberrations of the rodent eye by the contact lens.

8934-117, Session PMon

Gold nanorods: circular depolarization response and its use for contrast enhancement

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Optical coherence microscopy is a powerful imaging method with the advantages in terms of imaging depth, resolution and sensitivity. Because of these advantages OCM is a widely used imaging method for various applications. OCM is essentially a structural imaging modality. To extend its application for molecular imaging various contrast agents are available. Among various contrast probes, gold nanoparticles are widely used molecular contrast probes as gold nanoparticles provides tunable high scattering/absorption cross section due to Plasmon resonance and they are biologically inert. Because of these advantages gold nanoparticles are widely used molecular contrast probes in optical imaging methods. The problem with the conventional contrast probe is difficulty in differentiate a signal of gold nanoparticles from the surrounding background tissue thus even though gold nanoparticles are widely used contrast probes they offer poor contrast.

In our work we make use of the fact that very few biological tissues are known to provide a strong circular depolarized scattering signal. Asymmetric gold nanostructures such as gold nanorods have strong

circular depolarization signal. We propose to use such circular depolarization response along with a dark-field circular depolarization sensitive OCM system to enhance contrast. We will discuss the implementation of dark field circular depolarization sensitive OCM setup. Experimental results in a tissue phantom setup and cell will be presented to demonstrate efficient background reduction leading to enhancement in the contrast. We believe this enhancement in contrast can improve sensitivity in molecular detection using OCM.

8934-118, Session PMon

Dynamic analysis of mental sweating of eccrine sweat glands for various sound stimulus by optical coherence tomography

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We recently demonstrated the dynamic OCT for in vivo observation of physiological functions of small organs such as eccrine sweat glands and peripheral vessels under the human skin surface. In our previous work, the dynamic OCT analysis of mental sweating of a single eccrine sweat gland was made using the time-domain OCT (TD-OCT). A lot of eccrine sweat glands align along the hill of fingerprint on human fingertips with the density of several hundred glands in cm2.

By use of the fast speed swept-source OCT (SS-OCT), we demonstrate the dynamic OCT possible for a few tens of eccrine sweat glands. In this paper, we demonstrate dynamic analysis of mental sweating of a few tens of eccrine sweat glands on a human fingertip by optical coherence tomography. We propose a novel method for evaluation of the amount of excess sweat in response to mental stress, where the en-face OCT images of the spiral lumen of the eccrine sweat gland are constructed by data acquisition of the 128 B-mode OCT images. The dynamic analysis of mental sweating is performed by the time-sequential piled-up en-face OCT images with the frame spacing of 3.3 sec. It is found that the amount of sweat in eccrine sweat glands is significantly increased in proportion to the strength of the sound stimulus.

8934-120, Session PMon

Hann, Gaussian, and super-Gaussian window functions for reducing side-lobes in spectral domain optical coherence tomography

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In this study, we demonstrated the point spread functions (PSFs) after fast Fourier transform (FFT) with applying various window functions. We used a superluminescent diode (SLD) with a spectrum of a flat-topped intensity profile as a light source of SD-OCT. Although the Hann window function and the Gaussian window function could reduce the side-lobes, those occurred that the axial resolutions were broadened. In this manuscript, we suggested the super-Gaussian window function. The first side-lobe at the PSF with applying the super-Gaussian window was larger than it at the PSF with applying the Hann window or the Gaussian window. However, the second side-lobe at the PSF with applying the super-Gaussian window was similar to the second side-lobes of both windows. In addition, the axial resolution at the PSF with applying the super-Gaussian window was enhanced compared with it at the PSF with applying the Hann window or the Gaussian window.

8934-121, Session PMon

Adaptive compressed sensing for spectral-domain optical coherence tomography

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Spectral-domain optical coherence tomography (SD-OCT) is a non-invasive medical diagnosing technology. SD-OCT instrumentation uses low coherence light source and spectrometer to measure pathological changes of biological tissues. Since the detecting depth of OCT mainly depends on the resolution of spectrometer, CCD with huge number of pixels is prone to be used in OCT, enhancing the hardness of data transmission and storage. Compressed sensing (CS) can reconstruct image with data sampling rate far lower than that of Nyquist law, and has been widely used in medical imaging processing, such as CT, MRI, DOT and so on. The usage of CS in OCT could decrease the data output by spectrometer and reduces the trouble of large data transfer and storage, thus eliminating the complexity of processing system. The traditional CS uses the same sampling model for SD-OCT images of different tissue, leading to reconstruction images with different quality. We proposed a CS with adaptive sampling model. The new model is based on uniform sampling model, and the interference spectrum of SD-OCT is considered. The distribution of interference spectrum is used as weight to adjust the local sampling ratio in uniform sampling model. Compared with traditional CS, adaptive CS can modify the sampling model for images of different tissue according to different interference spectral, getting high quality reconstruction images without changing sampling model. In this paper, several biological samples are used to compare the reconstruction image quality and the results proved the advantages of adaptive CS.

8934-122, Session PMon

Optical teardown of a Kindle Paperwhite display by OCT

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An optical teardown, or reverse engineering, of an Amazon Kindle Paperwhite e-book reader display demonstrates an industrial application of Optical Coherence Tomography. The “teardown” was performed by Optical Coherence Tomography at 1310 and 1060 nm, utilizing two types of advanced data acquisition boards with Camera Link and PCIe interfaces. The PCIe version was used to stream 820,000 A-lines at a 100 kHz rate directly to PC memory. This data was used for an uninterrupted phase sensitive measurement of the electrophoretic display’s pigment motion using a new phase unwrapping algorithm that is tolerant to laser phase jitter.

The Kindle Paperwhite display incorporates an optical diffuser, lightguide and scattering layers for white light illumination, capacitive touch sensing, and an electrophoretic display. All these layers were imaged by OCT as well as the thin film transistor array on the back side for driving the pixels. Phase sensitive OCT is used to measure motion of the pigment particles as the display changes between black and white states, and shows that even though the display latches into a permanent visual state, the pigment particles are still moving through Brownian motion.

8934-123, Session PMon

Over-depth artifacts elimination in spectral-domain optical coherence tomography

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An efficient technique of correction of coherence gate curvature in spectral-domain OCT is proposed. A method of constructing of different shapes of single spectral component envelop is described. The control of the single spectral component envelop allows to eliminate over-depth artifacts caused by preserved partial coherence in the optical delays longer then coherence length.

8934-124, Session PMon

Speckle reduction in optical coherence tomography images via dynamic infinite-impulse-response filtering

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We present a temporal averaging method based on infinite-impulse-response (IIR) filtering to reduce speckle in OCT images. This method works in a recursive way, involving only two B-scan image frames to generate a filtered image. Thus, it performs with less computational complexity and time, unlike other conventional software-based approaches. To optimally reduce speckle noise while avoiding image blurring due to sample motion, the filter coefficient is dynamically determined, depending on parameters regarding motion detection and image uniformity. In this study, we tested the method during real-time operation via CPU processing. We used the mean-squared error (MSE) between two successive frames as a criterion to detect sample motion and changed the filter coefficient when the MSE exceeded a certain threshold to prevent image blurring. The optimal coefficient and motion detection threshold were chosen for robust and unblurred imaging in our testbed configuration. Results in our and conventional schemes are compared using various image quality metrics and via image observation. In our scheme, a higher filter coefficient gives better speckle reduction. In a conventional scheme averaging multiple frames, however, up to 20 frames should be averaged to achieve the similar performance. This requires more computational complexity and time. It was evident that in our scheme, the speckle reduction performance was quite promising and the fine detail of sample structures were preserved even with sample motion.

8934-125, Session PMon

Dual-fiber OCT measurements

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Conventional optical coherence tomography (OCT) usually requires complex optics at the tip of the OCT probe for focusing and scattered light collection. Such complexity is not compatible with common path OCT where the reference arm is replaced by the reflection from the probe fiber-air interface and the sample should be in close proximity to the fiber tip to have sufficient measurement depth. Indeed, separating the light injection and collection paths simplifies the OCT head design and allows integrating the scanner with the optical injection unit. In this work, we propose a new probe configuration that allows such separation in the OCT head. For this purpose, a swept source OCT setup was built, using a tunable laser (1486 nm to 1570 nm) with a 0.3 mW output power that was injected through a single-mode fiber (or a fiber with a scanner unit) and the scattered light was collected using another multi-mode fiber (MMF). The usage of the MMF in the reception enables higher power-collection efficiency and transfers the reference position to be at the first layer of the sample under test, which extends the theoretical maximum depth analyzed by the OCT system and overcomes the restriction of close proximity mentioned above. This simple concept has been validated by experimental measurement in the lab and has shown better results when compared with the traditional single-fiber common path OCT configuration. The experiments have been carried out on 1 mm-thick sheets of glasses with and without a MEMS scanner. Results for different scanning angles all show the sample under test's surfaces reflections at the expected correct positions.

8934-126, Session PMon

Monitoring of hemodynamic signals post optogenetic stimulation via optical coherence tomography

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Understanding neural and cerebral vasculature coupling is essential to the interpretation of functional brain imaging. We propose a new technique to stimulate and monitor the cortical microcircuits via Optogenetics and Spectral Domain Optical Coherence Tomography (SD-OCT) to measure neurovasculature coupling in-vivo. Optogenetics is a new methodology that has the ability to both stimulate and suppress neural activity with light in genetically targeted cells in the brain. SD-OCT is a non-invasive imaging technique suitable for imaging cortical microvasculature with high spatial and temporal resolution. Our proposed technique incorporates optogenetic technology into an imaging modality capable of recording hemodynamic variations. Using this platform to study changes in cerebral microvasculature, we have observed transitions in hemodynamics in correspondence with optogenetic stimulation. Transitions have been detected in continuous monitoring of blood flow and velocity before, during and after optogenetic stimulation. Along with changes in velocity, blood vessel dilation and constriction have been observed during stimulation using label-free SD-OCT angiography. The combined stimulation and imaging technique provides a versatile instrumentation for studying neural vascular coupling. Micro scale functional brain imaging based on neurovasculature monitoring modalities, such as the proposed system, provide new opportunities in future studies of neural-hemodynamic pathologies, such as epilepsy, cerebral infarction, and aphasia.

8934-127, Session PMon

Design consideration and performance analysis of OCT-based topography

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Optical coherence tomography is a multidimensional ranging techniques built on the principle of low coherence interferometry (LCI) that is capable of 2D and 3D subsurface and hence thickness topography in many biological samples with somewhat well-defined layers. Here, we report an in-depth study on design consideration and performance analysis of OCT-based topography by tracking of maximum intensity at each layer's interface. We demonstrate that, for a given stabilized OCT system, a high precision and accuracy of OCT-based layers and thickness topography in the order of tens nanometer can be achieved by using a technique of maximum amplitude tracking. The submicron precision was obtained by over sampling through the FFT of the acquired spectral fringes but was eventually limited by the system stability. Furthermore, we report some measurement techniques to characterize a precision, repeatability, and accuracy of the surfaces, sub-surfaces, and thickness topography using our optimized FD-OCT system. We verified that for a given stability of our OCT system, precision of the detected position of signal's peak of down to 20 nm was obtained. In addition, we quantified the degradation of the precision caused by sensitivity fall-off over depth of FD-OCT. The measured precision is about 20 nm at about 0.1 mm depth, and degrades to about 80 nm at 1 mm depth, a position of about 10 dB

sensitivity fall-off. The measured repeatability of thickness measurements over depth was approximately 0.04 microns. Finally, the accuracy of the system was verified by comparing with a digital micrometer gauging.

8934-128, Session PMon

Validation of a new real-time in-situ optical coherence tomography with modified oral probe by comparing with the certified CE marking optical coherence tomography dermatology probe

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A non-standard, non-CE certified recently modified optical coherence tomography (OCT) oral instrument, was calibrated and validated against the same manufacturer's standard commercial CE certified OCT skin instrument. Comparison between both instruments was achieved by measuring National Physical Laboratory (NPL) optical dimensional standards and metric slip gauges in X, Y, and Z-axes,?i.e. 3-D object measurement. Further, calibration of step height model (Z axis) and planar slope phantoms, crossing from air to aqueous media, were compared to investigate relative image behaviour across changing refractive index environments. Both instruments were also investigated utilising porcine cutaneous and subcutaneous tissue to investigate behaviour in dissimilar tissue types.

Results of the comparisons demonstrated that the new OCT oral system performed in a similar fashion to the CE certified OCT skin instrument and can be used as a surrogate in measurement of cutaneous and oral tumour thickness and depth with confidence and accuracy.

8934-129, Session PMon

Photodynamic therapy induces epidermal thickening in hairless mice skin: an optical coherence tomography assessment.

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Photodynamic therapy (PDT) promotes skin improvement according to many practioners, however the immediately in vivo assessment of its response remains clinically inaccessible. As a non-invasive modality, optical coherence tomography (OCT) has been shown a feasible diagnostic technique that provides optical imaging in real time, avoiding tissue biopsies. For this reason, our investigation focused on evaluates the PDT effect on a rodent model by means of OCT. Therefore, a normal hairless mouse skin has undergone a single session of PDT, which was performed with topical 5-aminolevulinic acid (ALA) cream using a red (630 nm) light emitting diode (LED) and reaching the dose of 75 J/cm2. As the optical imaging tool, an OCT (930 nm, Thorlabs Inc) with axial resolution in air of 6.0 microns was used, images with 2000x512 pixels was acquired with no-contact to sample at 4 frames per second. Our result demonstrates that, within 24 hours after the PDT procedure, the mouse skin has shown epidermal thickness, which has gradually increased 2 weeks apart the PDT procedure. Moreover, the skin surface has become flattened after PDT. Concluding, this investigation demonstrates that the OCT is a feasible and reliable technique that allows real-time cross-sectional imaging of skin, which can predict whether the treatment reaches its goal.

8934-130, Session PMon

Temporal analysis of optical coherence tomography to measure glucose levels in blood

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It is hypothesized that Optical Coherence Tomography combined with Dynamic Light Scattering techniques can be used to accurately determine glucose levels in blood.

Diabetes is a common disease in which patients experience dramatic swings in blood glucose concentrations. Patients are required to prick their finger at least three times daily to determine their blood glucose level, and report significant levels of discomfort with this invasive method. Despite the high clinical need, a non-invasive method to quantify blood glucose levels does not exist.

Dynamic light scattering (DLS) is a useful tool for measuring translational and rotational dynamics of scattering microparticles in suspension. The phase and amplitude of the scattered light field are modulated by the dynamics of scattering particles. Recoding the resulting intensity fluctuations as a function of time using a photo-detector can yield certain physical properties of the scattering particles (their effective sizes / diameters) and the suspending fluid (its viscosity).

In an optically dilute suspension, light scatters only once, and detailed information about the sample, such as effective scatterer diameter or viscosity, can be obtained. In optically dense samples such as most biological tissues, however, some of the light scatters multiple times before detection, causing significant information loss. Therefore, it is desirable to have an optical technique that can separate singly from multiply scattered light, thus permitting detailed medium characterization even within optically dense media. Optical Coherence Tomography (OCT) is one such optical technique.

8934-131, Session PMon

Development of real-time dual displaying handheld and bench-top hybrid mode SD-OCT

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We demonstrated a dual displaying handheld optical coherence tomography (OCT) is developed to diagnose volunteer's retina and optic nerve head without any constraint of the volunteer's motion. The developed system is a portable and easily movable system as it contains the compact-portable OCT system including the handheld probe and computer. The volunteer posterior chamber of the eye can be diagnosed by using the handheld probe and also the handheld probe can be fixed to the bench-top cradle if the volunteer is not in a normal physical condition. The image displaying of this handheld probe can be done using the computer monitor and the inbuilt secondary small monitor in real-time display, and also without any necessity to view the computer monitor the real-time displaying images can be saved by using the inbuilt button of the Handheld probe. The large scale signal processing procedures such as, k-domain linearization, Fast Fourier Transform (FFT) and log scaling signal processing can be done rapidly by using graphics processing unit (GPU) accelerated processing rather than central processing unit (CPU) processing. The pixels size of the Labview program based system is 1024 ? 512 and the frame rate is 56 frame/sec which can be used for the real-time displaying. The 3D images of the volunteer posterior chamber including retina, optic nerve head, blood vessels and optic nerve were composed by using real-time displaying images with a pixel size of 500 ? 500 ? 500. In this paper, handheld and bench-top hybrid mode with dual displaying handheld OCT was developed to overcome the drawbacks of the conventional method.

8934-132, Session PMon

Three-dimensional real-time displaying optical coherence tomography for diagnosis of human otitis media

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We report the application of Optical Coherence Tomography (OCT) to various types of human cases of otitis media (OM). Whereas conventional diagnostic modalities for OM, including standard and pneumatic otoscopy, are limited to visualizing the surface information of the tympanic membrane (TM), OCT is able to effectively reveal the depth-resolved microstructural below the TM with a very high spatial resolution. With the potential advantage of using OCT for diagnosing different types of OM, we examined in-vivo the use of 840 nm wavelength, and OCT spectral domain OCT (SDOCT) techniques, in several human cases including normal ears, and ears with adhesive and effusion types of OM. Peculiar positions were identified in two-dimensional OCT images of abnormal TMs compared to images of a normal TM. Analysis of A-scan (axial depth-scans) data from these positions could successfully identify unique patterns for different constituents within effusions. These OCT images may not only be used for constructing a database for the diagnosis and classification of OM, but they may also demonstrate the feasibility and advantages for upgrading the current otoscopy techniques.

8934-133, Session PMon

White light low coherence interferometry for wavelength dependent refractive index measurement of biological cells and tissues

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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 obtained from phase-based analysis. Quantitative phase imaging (QPI) techniques allow us to quantitatively distinguish between cancerous and non cancerous cells and tissues. We present white light quantitative phase microscopy with color fringe analysis for QPI and determination of wavelength-dependent refractive index of cancerous and non cancerous cells and tissues using phase-shifting and single chip colour CCD camera. Five frame phase-shifting methods were used and phase-shifted white light interferograms are recorded by single chip colour CCD camera. Thus the data acquisition procedure is similar to single wavelength interferometry. Individual interferograms of red, green and blue colour are then separated from each phase-shifted interferogram and processed. The present technique does not require multiple colour laser sources, spectral filters and dispersive optical elements to quantify wavelength dependent phase-maps and refractive index. It uses a low cost white light source, a conventional optical microscope, a nearly common path Mirau-interferometric objective lens and a low cost colour CCD camera. Present technique might provide important insight for non-invasive determination of refractive index variation with wavelength within cells and tissues with ease of operation and low cost.

8934-29, Session 5

Shear wave elastography using phase sensitive optical coherence tomography

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Optical coherence tomography (OCT) provides high spatial resolution and sensitivity that are ideal for imaging the cornea and the intraocular lens. Quantifying the biomechanical properties of these tissues could add clinically valuable information. Thus, we propose a dynamic elastography method combining OCT detection and a mechanical actuator to map the shear modulus of soft tissues.

We performed experiments on agar phantoms and in vivo mouse corneas. Shear waves were induced using a piezoelectric actuator in contact with the sample and driven by a 5 kHz burst with five micron amplitude. The sample was imaged during shear wave propagation with a phase-sensitive OCT system operating in M-B mode at an equivalent frame rate of 47 kHz. Phase differences between M-scans yield axial displacements over time. The local shear wave speed is then mapped using a time-of-flight algorithm.

We obtained shear wave speed maps in a 0.5% agar phantom containing a 1% agar inclusion. The shear wave speed in each region was estimated to be 2.10 ± 0.07 m/s and 4.3 ± 0.4 m/s respectively, consistent with literature values. We also obtained shear wave speed maps of corneas in anesthetized mice.

We demonstrated the feasibility of using OCT to perform dynamic elastography providing high-resolution and quantitative measurements for both in vitro and in vivo conditions. Animal models of corneal pathologies will be examined to support the clinical potential of our method. The use of non-contact shear sources is also being investigated for clinical translation.

8934-30, Session 5

Quantitative two-dimensional micro-displacement measurement by optical coherence tomography

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There are several applications of quantitative micro-displacement measurement of a biological specimen, including characterization of mechanical property and monitoring a laser-induced photothermal expansion. In this study, we proposed a new quantitative micro-displacement measurement method using optical coherence tomography (OCT). Specifically, the axial displacement is measured by Doppler OCT and lateral displacement is measured by correlation analysis of OCT signal amplitude. The distinctive properties of our proposed method are followings: First, the method provides high accuracy for the sample which has heterogeneous optical properties. Second, this is useful for the very small displacement. Third, the modified correlation coefficient is robust to the effect of noise. By using this method, we measured the local and micro-displacement of the part sclera. In this measurement, a local displacement, i.e. deformation, is induced by pushing the sample by a piezoelectric transducer. A custom built swept-source OCT is used for the measurement. A sequence of B-scans is taken at the same position, and the displacement analysis was performed between B-scans before and after the pushing. Then, the estimated micro-displacement is represented by the magnitude and orientation. The results showed that the almost all of the part sclera is moved axially, in contrast, the lateral displacements were found to be more localized than the axial displacements. This might indicate the heterogeneous mechanical property of the tissue. In conclusion, we demonstrated the new quantitative micro-displacement measurement, and the method successfully visualized spatially resolved micro-displacement distribution of a biological tissue.

8934-31, Session 5

Resonant acoustic radiation force optical coherence elastography

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Materials respond primarily at their mechanical resonance frequencies. These frequencies infer the elastic properties and the geometry of the materials. A resonant acoustic radiation force optical coherence elastography method utilizing the mechanical resonant frequency to effectively distinguish tissues of varying stiffness is presented in this paper. This method offers noncontact, noninvasive, ultrahigh displacement resolution and real-time evaluation of elastic properties from direct measurement of sample resonance. A focused ultrasound transducer was used to remotely excite the sample to generate a local vibration at the step-driven frequencies and the phase-resolved Doppler optical coherent tomography measurement was employed to measure the resonant frequency of the sample. First, the linear dependency of the resonant frequency on the square root of Young's modulus was validated on silicone phantoms with different mechanical properties. Second, both the frequency response spectrum and the 3D imaging results of an agar phantom with a hard inclusion further confirmed the feasibility of deploying resonant frequency method for stiffness prediction. Furthermore, we performed experiments using this resonant ARF-OCE method on a section of post-mortem human coronary artery. The results of the current study demonstrate the capability of the resonant ARF-OCE method as a non-invasive assessment of pathological tissue with the potential for its use in clinical settings in the future.

8934-32, Session 5

Phase-sensitive optical coherence tomography in the middle ear using an akinetic swept laser source

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Hearing loss is a common sensory problem observed in all age groups with various risk factors, and causes critical impairment to the quality of life. Auditory processing in the middle ear changes sound waves to a mechanical vibration by the movement of the tympanic membrane (TM) and the ossicles. Monitoring the morphological structure and mechanical vibration of the middle ear is a crucial diagnostic approach for conductive hearing loss. Phase-sensitive OCT can visualize the morphological structures of the middle ear and measure its mechanical vibration. We developed a fiber-based phase-sensitive OCT system with an akinetic swept laser source for the measurement of mechanical vibration in the middle ear. The akinetic swept laser is electronically tuned and precisely controls sweeps without any mechanical movement, which results in minimal phase instability. The swept source was operated with a wavelength 1550 nm, sweep rate of 140 kHz, and provided picometer-scale phase sensitivity. The phase instability of the swept source was evaluated with a common-path interferometry setup. Utilizing phase-sensitive OCT, we acquired the structures and vibrations of the middle ear of an ex vivo mouse model with high-speed real-time performance with field programmable gate array (FPGA) architecture.

8934-33, Session 5

Depth-resolved detection of tissue biomechanics for optical coherence elastography of crystalline lens

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Noninvasive assessment of the biomechanical properties of crystalline lens in situ is required in order to gain the understanding and improve the diagnosis and therapy of the accommodation-related ocular diseases. However, the transparency, the location and the inhomogeneity of the crystalline lens make it challenging to perform elastographic measurements using current techniques. Here, we report results of using focused ultrasound excitation combined with optical coherence tomography (OCT) to assess depth-resolved mechanical properties of transparent samples simulating crystalline lens. The method applies focused ultrasound waves to remotely interrogate the sample surface. Phase-sensitive OCT technique is used to measure the induced surface displacement over time with the sensitivity at nanometer scale. Spectral analysis of the temporal profiles of elastic waves is performed to study the dynamics of the sample surface after the external stress is removed. From the amplitude information of the frequency spectrum, the natural frequency of the sample is obtained and is used as the indicator of the mechanical properties of the sample. The pilot experiments were performed on homogeneous and layered tissue-mimicking phantoms. It is indicated that the layers from different depths introduce different frequencies to the surface response, and the results demonstrate the feasibility of the proposed method in depth-resolved detection of the biomechanics in transparent samples. Also, our preliminary studies on ex vivo bovine crystalline lens suggest the potential of this nondestructive method for in situ elastographic measurement of the depth-dependent mechanical properties of crystalline lens.

8934-34, Session 5

Nano-sensitive optical coherence tomography (nsOCT) for depth resolved characterization of 3D submicron structure

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Optical coherence tomography (OCT) is a rapidly developing technique with various applications, including biomedical imaging and diagnosis. One of the main shortcomings of current OCT techniques is low resolution and sensitivity to structural changes (typically about 10 microns). The best ultra-high resolution OCT techniques demonstrate sensitivity to structural changes and depth resolution of about 1 micron. Since many applications of interest (such as cancer) require detection of structural changes on the nanoscale, OCT would definitely benefit from improved structural resolution and sensitivity.

A new spectral encoding of spatial frequency (SESF) approach for quantitative characterization of the structure with nanoscale sensitivity has been developed recently. The ability to map axial structural information into each pixel of a 2D image with nanoscale sensitivity has been demonstrated and application of this approach to 3D microscopic imaging has been discussed.

Here we present a novel technique, nano-sensitive OCT (nsOCT), to dramatically increase sensitivity of the OCT to structural changes. Together with reconstruction of the conventional 3D OCT image we propose to directly translate information about the particular structure

from the Fourier domain to the image domain and map this information into the corresponding location within the 3D image. As a result, submicron axial structure can be visualized and nanoscale structural alterations within each voxel of the 3D OCT image can be detected. Preliminary results show that using nsOCT, based on conventional spectral domain OCT system with resolution 12 ?m x 30 ?m x 30 ?m, it is possible to detect structural changes within scattering sample as small as 20 nm.

8934-35, Session 6

MAPS-OCT contrasts diffusing nanorods and cellular motility in 3D mammary epithelial cultures

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Optical coherence tomography (OCT) has gained increasing application in providing sufficient speed and depth-resolution to image 3D tissue cultures. Here we propose a new method for contrasting the locations of diffusing gold nanorods introduced into the artificial extracellular matrix (ECM) of a mammary epithelial cell (MEC) tissue culture. Temporal and polarization properties computed from a series of polarization-sensitive OCT images are analyzed to quantify the amplitude and rate of speckle fluctuations (effective motility and autocorrelation, respectively), and the normalized cross-polarization. In this system, each class of light scattering objects within the sample (gold nanorods, MECs, and ECM), exhibit a unique Motility-, Autocorrelation-, and Polarization-Sensitive (MAPS) signature. Rendering MAPS-OCT images for each signature is shown to be greater than the sum of its parts, providing greater sensitivity and specificity to the locations of gold nanorods and MECs than any of the contrast metrics alone. Using MAPS-OCT, we found that PEGylated, diffusing gold nanorods fully permeated the ECM, but remained spatially separated from MEC spheroids up to 1 day after topical application to tissue culture.

8934-36, Session 6

Detection of pH-induced aggregation of smart gold nanoparticles with photothermal optical coherence tomography

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We demonstrate the detection of pH-induced aggregation of a novel photothermal contrast agent so called as “smart” gold nanoparticles (AuNPs) by using photothermal optical coherence tomography (PT-OCT). “Smart” AuNPs are 10 nm in size and become photothernal active only with aggregation. They are designed to form aggregation in mild acidic condition and can be targeted to cancerous cells and tissues. A PT-OCT system was developed by combining a wavelength swept source with 50 kHz axial scanning rate at 1310nm for OCT and a 660 nm diode laser for photothermal excitation. Parameter characterization and PT-OCT system sensitivity was done. Experiment was first monitored with solution samples at two different pH conditions. Optical path length (OPL) variation increase was detected as a funtion of time in mild acidic condition, while not much changes in neutral condition. Further experiment was done with two cell specimens: Hela and fibroblast cells as cancer and normal cells respectively. Dark-field microscope images were taken to check the aggregation level of “smart” AuNPs. The images showed that the “smart” AuNPs aggregated in HeLa cell sample during the incubation while no apparent aggregation in NIH3T3 cell sample. PT imaging was applied to both samples in order to detect the aggregation of “smart” AuNPs by the PT-OCT system. Elevated OPL variation signal was detected with Hela cell specimens due to low pH condition. These results have shown the detection of pH-induced aggregation of “smart” AuNPs in acdic condition by PT-OCT. With the novel optical property of “smart” AuNPs and the noninvasiveness as well as high sensitivity of PT-OCT, this technique is promising for targeted cancer detection.

8934-37, Session 6

In vivo imaging of gold nanorod delivery to tumors using photothermal optical coherence tomography

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Gold nanoparticles are currently under development for clinical applications in cancer imaging and therapy due to their attractive biological, chemical, and optical properties. Noninvasive imaging of the uptake kinetics and distribution of gold nanoparticles in tumors in vivo would enable robust evaluations of nanoparticle properties (e.g. nanoparticle size, shape, and surface chemistry) for improved delivery. Here, we demonstrate photothermal OCT (PTOCT) as a tool for tracking gold nanoparticles in vivo with high resolution and wide field of view in three dimensions. Gold nanorods (AuNRs, 200 µl, 9 nM) were systemically injected into nude mice with 4T1 mammary tumors implanted into dorsal skinfold window chambers. PTOCT, performed pre-injection and 2, 16, and 24 hours post-injection, identified the presence of AuNRs due to the enhanced permeability and retention of tumors. AuNRs caused a significant increase ($p<0.05$) in PTOCT signal at all time points after injection compared to pre-injection. The maximum PTOCT signal due to AuNR accumulation occurred 16 hours after injection (106?45 picometer optical path length photothermal oscillations, n=4 mice). Tumors were then harvested and imaged ex vivo with multiphoton microscopy as an independent validation of AuNR uptake. Multiphoton luminescence signals significantly increased ($p<0.05$) for tissues from AuNR-injected mice compared to negative controls. We have shown that PTOCT can monitor the distribution of gold nanoparticles as they passively accumulate in tumors in vivo. In the future, PTOCT could be used to optimize in vivo drug delivery schemes for gold nanoparticles.

8934-38, Session 6

Photothermal optical coherence tomography based on localized surface plasmon resonance enhanced absorption of Au nanoring

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Compared with other Au NPs, an Au nanoring (NRI) has more geometry parameters for controlling the localized surface plasmon (LSP) resonance wavelength. In particular, it can extend the LSP resonance wavelength into the range between 1000 through 1300 nm, in which tissue absorption and/or scattering can be weaker such that light penetration can be deeper. Also, an Au NRI can be designed for their scattering cross section and absorption cross section to be comparable such that broader imaging application can be feasible. In this paper, we use the LSP resonance around 1300 nm of Au NRI to demonstrate the photothermal optical coherence tomography (OCT) operation. In particular, we compare the scanning results of two OCT systems with the operation wavelengths at 1310 and 800 nm. In the scanning operation of the 1310-nm OCT system, both enhanced scattering and absorption of LSP resonance are involved in the OCT scanning results. However, in the scanning operation

of the 800-nm OCT system, only enhanced absorption of LSP resonance is involved in the scanning results. Phantoms with agar background and mixed Au NRI solution are used as OCT scanning samples. Bean milk of various concentrations is mixed into agar for varying the background scattering levels. It is found that although the intensity image signal is weak with the 800-nm OCT system, its photothermal signal is quite strong, particularly when bean milk is mixed with agar for enhancing background scattering. The photothermal signal level increases with increasing background scattering when the OCT operation wavelength is located outside the spectral range of LSP resonance of Au NRI.

8934-39, Session 6

In vivo molecular contrast OCT imaging of methylene blue in a zebrafish embryo

Wihan Kim, Brian E. Applegate, Texas A&M Univ. (United States)

Developing molecular contrast for Optical Coherence Tomography (OCT) holds the promise of micron scale resolution molecular imaging at depths up to 2 mm. In particular, the imaging of FDA approved dyes such as methylene blue (MB) could lead to more rapid adoption for clinical applications since the regulatory burden would be substantially reduced. We have introduced a 663 nm diode laser into an otherwise typical 830 nm spectral-domain OCT system by inserting a dichroic mirror into the sample arm of the OCT system. This relatively simple and inexpensive modification has enabled in vivo imaging of MB in a zebrafish embryo. The embryo was stained by immersing it in a 0.01% solution of MB for 6 hours. For reference, sentinel lymph node identification using MB prior to breast cancer surgery requires the injection of a 1% MB solution. In vivo images were acquired with a total power on the sample of 2.8 mW split equally between the pump and probe, well below the ANSI limit for skin. As expected, volumetric images show accumulation of MB in the mesonephros, which is the primary excretory organ. Gaining molecular contrast in OCT images from an FDA approved dye such as MB could find use both as a research tool and clinically to enhance the contrast of OCT images.

8934-40, Session 6

Dual wavelength-band spectroscopic optical frequency domain imaging using coherent scattering in metallic nanoprobes

Tae Shik Kim, Sun-Joo Jang, Nuri Oh, Yongjoo Kim, Taejin Park, Ji Ho Park, Wang-Yuhl Oh, KAIST (Korea, Republic of)

Optical frequency domain imaging (OFDI) is one of the second-generation optical coherence tomography (2G-OCT) techniques [1,2]. Because of its high-speed, high-sensitivity, cross sectional and depth sectioning imaging capabilities, OFDI has been considered as a promising imaging tool for biological studies as well as medical and clinical applications [3,4]. However, conventional OCT only provides the intensity information which is proportional to the amount of scattered light from the sample. As one approach to extract additional complementary information, spectroscopic OCT (SOCT) techniques with either endogenous or exogenous contrast agents are suggested [5-7]. While spectroscopic imaging with endogenous agents has an advantage that it is not necessary to inject additional materials into the sample, in many cases the spectroscopic contrast from endogenous agent is not sufficiently strong. Since OCT is a coherence-based imaging technique, it is impossible to utilize fluorescent dyes as contrast agents. In this presentation, we demonstrate dual wavelength-band spectroscopic optical frequency domain imaging (OFDI) using coherent plasmon resonant scattering from the metallic nanoparticles as exogenous spectroscopic contrast agents. A combination of two different-sized nanoparticles and a dual wavelength-band OFDI system whose wavelengths match to the plasmon resonant scattering wavelength of each nanoparticles, shows clear differentiation and visualization of the locations where each nanoparticles exists in a scattering phantom and in biological tissue in-vivo.

8934-41, Session 7

Visible light optical coherence tomography for retinal oximetry

Ji Yi, Qing Wei, Wenzhong Liu, Hao F. Zhang, Northwestern Univ. (United States)

Although retinal blood oxygen saturation (sO2) is a vital physiological parameter for various retinal related diseases, the quantification of sO2 have been overlooked mostly due to lacking a precise tool. The multi-wavelength fundus photography have been the prevalent method to calculate sO2. However, the precision of this methods has been undermined by several confounding factors such as reflection of the blood vessels surface, scattering from the neighboring tissue, effect of melanin etc. The fundamental limitation of fundus photography is that it cannot provide depth discrimination of the light signals. On the other hand, optical coherence tomography provides three dimensional retinal structure, and the broadband illumination provided a way to investigate spectral behavior at a particular location.

Here we applied visible light optical coherence tomography to in vivo quantify retinal sO2. The spectral analysis was performed at the bottom blood vessel walls so that both absorption and scattering contrast from hemoglobin can be modeled and utilized. The packing factor considering the multiple scattering effect of densely packed red blood cells were considered in our model and weighted the scattering spectrum. By this comprehensive model, we have demonstrated the capability of quantifying sO2 from individual blood vessels and found that the optimal fitting can be achieved when the packing factor is equal to 0.2. Also, due to the strong absorption of the blood in visible light range, the retinal microvasculature can also be enhanced.

8934-42, Session 7

Stability in computed optical interferometric tomography for in vivo imaging

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Stability is a fundamental requirement for many imaging modalities. Whether it is stability of the overall imaging system, or stability of the sample being imaged, knowledge of specific stability requirements and techniques to characterize stability is of utmost importance. This paper explores the stability requirements for phase sensitive techniques such as interferometric synthetic aperture microscopy (ISAM) and computational adaptive optics (CAO). In addition, we set forth a method to determine the three-dimensional system or sample stability, which is important for a large range of phase-sensitive imaging modalities including Doppler or phase variance optical coherence tomography. Both tissue phantom and in vivo tissue imaging help to identify fundamental stability requirements for ISAM/CAO as well as to demonstrate the feasibility of these results in practical imaging applications.

8934-43, Session 7

Real-time speckle reduction using wavefront modulation in multi-functional optical coherence tomography images

Christian M. Oh, Koji Hirota, M. Rezuanul Haque, Univ. of

California, Riverside (United States); Yan Wang, Harvard Medical School (United States) and Massachusetts General Hospital (United States); M. Shahidul Islam, B. Hyle Park, Univ. of California, Riverside (United States)

The presence of coherent speckle in optical coherence tomography (OCT) images can obscure identification of small or thin tissue structures. Intensity variation of speckle can blur boundaries between tissue structures with small differences in scattering properties, hindering visualization of small or faintly reflecting morphological features of clinical value. Recently proposed methods for speckle reduction have included digital filters applied during image postprocessing and physical techniques involving compounding of multiple acquisitions of the same area, including frequency and spatial compounding. Although these methods have led to promising results, their drawbacks involve computational intensive postprocessing and extensive system modifications which lead to sacrifice of SNR and resolution as well as lack of real time analysis. We present a method of reducing the effect of speckle in a multifunctional spectral domain OCT system by modifying the wavefront and consequently the speckle pattern using a deformable mirror placed in the sample arm beam path. Our results indicate that by modifying the wavefront between each depth profile acquisition and subsequently incoherently averaging adjacent depth profiles, we can achieve a considerable reduction in speckle contrast. We demonstrate the results of our technique on samples including bovine retinal nerve fiber layer & human skin (intensity), chicken muscle/tendon (polarization), and flowing intralipid solution (flow).

8934-44, Session 7

Depth-resolved attenuation coefficient estimation from beam-shape corrected OCT scans of phantoms

Koenraad A. Vermeer, Rotterdam Ophthalmic Institute (Netherlands); Jianhua Mo, Jelmer J. A. Weda, Vrije Univ. Amsterdam (Netherlands); Hans G. Lemij, The Rotterdam Eye Hospital (Netherlands); Johannes F. de Boer, Rotterdam Ophthalmic Institute (Netherlands) and Vrije Univ. Amsterdam (Netherlands)

We present a method to extract the attenuation coefficient of tissue from optical coherence tomography (OCT) depth scans. Attenuation coefficients are related to tissue properties. In contrast to the conventional OCT signal strength, these attenuation coefficients are invariant to the OCT beam's local intensity. They therefore provide a way to quantitatively assess the tissue's state while simultaneously reducing various imaging artifacts.

Previously, a model-based method was introduced to calculate the retinal nerve fiber layer (RNFL) attenuation coefficient, by using the retinal pigment epithelium (RPE) as a reference layer. We present a reference-free method to calculate the depth-resolved attenuation coefficient from OCT data, taking the axial point spread function (aPSF) of the focused OCT beam into account. Evaluation was done on phantoms, where the attenuation coefficients were controlled by varying the concentration of TiO2 scatterers. The new method was then compared to the conventional attenuation coefficient estimation from the slope of the OCT signal.

The system's focal point was estimated from the phantoms' volumetric OCT scans. Then, for each sample, a single B-scan was selected and the attenuation coefficient was estimated by both methods from the uncorrected data and the aPSF-corrected data. For small attenuation coefficients (<0.1 mm^-1), the OCT slope method deviated from the expected linear relationship between concentration and attenuation coefficient. The novel method maintained this relationship even for very small attenuation coefficients (0.01 mm^-1) if the OCT beam's aPSF was taken into account.

The presented method enables depth-resolved estimation of attenuation coefficients over a large, biomedically relevant range.

8934-45, Session 7

Improved attenuation coefficient and birefringence parametric optical coherence tomography imaging of burn scars using vasculature masking

Peijun Gong, The Univ. of Western Australia (Australia); Yih Miin Liew, Univ. of Malaya (Malaysia); Lixin Chin, Shaghayegh Eshaghian, Peter R. T. Munro, The Univ. of Western Australia (Australia); Fiona M. Wood, Royal Perth Hospital (Australia) and The Univ. of Western Australia (Australia); David D. Sampson, Robert A. McLaughlin, The Univ. of Western Australia (Australia)

Parametric optical coherence tomography (OCT) imaging is a method of extracting the optical properties of tissue from OCT scans. Parametric OCT forms images of tissue where the value of each pixel is related to a particular underlying optical property, such as attenuation or birefringence, of the tissue. It replaces the OCT image, which is relative in nature, with an absolute quantification of a tissue parameter by trading off axial resolution. When applied to the attenuation coefficient and/or birefringence for the assessment of burn scars, it is confounded by the presence of prolific vasculature which creates artifacts in these absolute measurements. We present a technique to automatically identify and mask blood vessels, removing their effects from the parametric OCT images. We present results on in vivo human burn scar tissue, and construct parametric OCT images for two parameters: the optical attenuation coefficient and birefringence. Using this technique, we quantify the difference between scar and normal skin tissue.

8934-46, Session 7

Intelligent microinjector for intracardiac microinjection based on common-path optical coherence tomography fiber sensor

Mingtao Zhao, Yong Huang, Jin U. Kang, Johns Hopkins Univ. (United States)

We describe a novel intelligent microinjector with a common–path optical coherence tomography (CP-OCT) fiber probe that can accurately inject drug at a specific site or layer with a resolution in the order of 10?m. The drug injection depth is maintained by a sensor controlled PZT motor with a step resolution of <1 ?m, which also significantly reduce the hand tremor of the surgeon using a close-loop PID control algorithm based on GPU computing. Biological tissue of swine heart was employed to test the performance of intracardiac microinjector. The lower hand tremor frequencies less than 4 Hz were clearly compensated by the system with a RSME of less than 12 micrometers, which is much less than that of free hand operation with RSME of 113 micrometers. 3-D visulizaiton of the milk injection inside the tissue was asessed using a 850nm SDOCT system. Such intelligent microinjectors can avoid localized hemorrhage and delicate tissue destruction at the injection site.

8934-47, Session 7

Imaging of the interaction of low frequency electric fields with biological tissues by optical coherence tomography

Adrián Peña Delgado, Jack Devine, Alexander Doronin, Igor V. Meglinski, Univ. of Otago (New Zealand)

Low frequency electric fields propagating in ex vivo biological tissues have been observed by using double correlation optical coherence tomography (OCT). An adaptive Wiener filtering approach has been

used to remove background noise, and a Fourier domain correlation algorithm has been applied to the sequence of OCT images. The results present the first direct observation of the scope of the electric field influencing biological tissues with OCT. The results show that variation in voltage and frequency of the applied electric field relates exponentially to the magnitude of its influence on biological tissue. The magnitude of influence is about twice more for fresh tissue samples in comparison to non-fresh ones. The obtained results suggest that OCT can be used for observation and quantitative evaluation of the electro-kinetic changes in biological tissues under different physiological conditions, functional electrical stimulation, and food quality control.

8934-48, Session 7

A computational model of optical coherence tomography employing an electromagnetic description of light

Peter R. T. Munro, Andrea Curatolo, Lixin Chin, Brendan F. Kennedy, David D. Sampson, The Univ. of Western Australia (Australia)

The most prominent theoretical models of optical coherence tomography are based upon the extended Huygens-Fresnel formalism, introduced to the community in the late nineties. These models have successfully predicted experimental results and have led to, for example, improvements in system design and optimisation, image interpretation and understanding of fundamental limitations of the technique. These models have been the subject of substantial enhancements in order to expand the range of system and sample parameters able to be simulated. However, there are some experimental conditions which are only able to be modelled by employing an electromagnetic description of light in both the optical system and sample. In this paper we present such a model, capable of calculating optical coherence tomography images of arbitrary, deterministic samples, using light sources of general states of coherence and general optical systems. We use the model to compare simulated images with experimentally obtained images of a three dimensional structured phantom.

We will employ this model to answer some fundamental questions regarding OCT imaging, including: What are the differences between OCT image formation using discrete particle phantoms and in biological tissue; where refractive index variations occur continuously? What are the intrinsic limitations of parametric imaging? What role does depolarisation play in image formation of biological tissue? What are the imaging properties of novel beam types such as Bessel beams?

8934-49, Session 8

OCT imaging of capillary RBC flux and speed

Jonghwan Lee, Weicheng Wu, Harvard Medical School (United States); Frédéric Lesage, Ecole Polytechnique de Montréal (Canada); David A. Boas, Harvard Medical School (United States)

As capillaries exhibit heterogeneous and fluctuating dynamics even during baseline, techniques measuring RBC flow properties over many capillaries at the same time will be very useful. Here, we report that dynamic OCT imaging can capture individual RBC passage since RBCs exhibit larger backscattering than blood plasma. We repeated B-scans at a cross-sectional plane of the cortex, and found a number of peaks representing RBC passage in the OCT intensity time courses at the voxels of capillary centers. This finding enabled us to quantify the RBC speed (mm/s), flux (RBC/s), and linear density (RBC/mm), simultaneously over many capillaries located at different depths. These measurements were validated by comparing with those mimicking the traditional two-photon line scanning technique. Compared to Doppler OCT, our technique identifies individual RBC passage and thus enables direct measurements of RBC speed and flux in capillaries even when they lay in the transverse direction.

Based on the above findings, we developed another technique for more rapid volumetric imaging of capillary network flow. Rapid volumetric imaging is required for functional studies where capillary flow dynamics should be imaged with 1-s temporal resolution. For this purpose, we defined a novel metric, statistical intensity variation (SIV), and validated that its mean averaged along a capillary path is proportional to RBC flux. SIV volume data was acquired by repeating only two B-scans, and then analyzed for vectorizing capillaries with flux estimations. This process enabled us to measure RBC flux over hundreds of capillaries with 1-s temporal resolution during functional activation.

8934-50, Session 8

Flexibly combined optical microangiography and dual-wavelength laser speckle system for comprehensive imaging of hemodynamic and metabolic responses

Lei Shi, Jia Qin, Lin An, Ruikang K. Wang, Univ. of Washington (United States)

We have proposed and developed a non-invasive biomedical optical imager combined from the subsystems of optical microangiography and dual-wavelength laser speckle contrast imaging. The system was designed to maintain the performances of both the subsystems. It was capable of simultaneously imaging the hemodynamic and metabolic responses in tissue environment in vivo. To achieve such requirements, we utilized unique optical designs, such as paired dichroic mirrors to compensate dispersion, additional relay lens to increase working distance and translational sample probe to select imaging area and focal plane freely. The multi-functionality of the system was demonstrated in a thorough investigation of hemodynamic and metabolic responses to an acute wound healing model in mouse pinna in vivo. The microvasculature, blood flow and hemoglobin concentration from millimeter level down to single capillary were comprehensively visualized. The captured instantaneous responses to wound onset showed great differentiation between areas in the pinna tissue; a rebalance tendency was exhibited in the following blood flow response and simultaneously a dynamic recovery to baseline situation was revealed in the hemoglobin concentration variation.

8934-51, Session 8

Wide-field and high-resolution mapping of network blood flow using optical coherence tomography

David G. Blauvelt, Yun-Sheng Chen, Xiaoxing Han, Brett E. Bouma, Wellman Ctr. for Photomedicine (United States); Rakesh K. Jain, Timothy P. Padera, Harvard Medical School (United States); Benjamin J. Vakoc, Wellman Ctr. for Photomedicine (United States)

There is a need for intravital vascular imaging tools that can simultaneously provide (i) resolution sufficient to resolve capillaries, (ii) imaging fields large enough to characterize network organization, and (iii) blood flow velocity quantification in every vessel within the network. OCT has been shown to provide satisfy the first two requirements, but comprehensive (voxel-by-voxel) blood flow mapping has not been possible within reasonable imaging times. Here, we present a flow mapping approach that combines a novel statistical method to extract blood flow from OCT intensity signals with a complex beam scanning design. We demonstrate that the resulting instrument is able to measure flow in each voxel of a 1000x1000x1000 voxel field in imaging times of 10-20 minutes. We validate this method by comparison with multiphoton-

based flow measurements, and demonstrate its utility in studies of cancer including short and long-term longitudinal measurements of blood flow within a tumor, and measurement of blood flow changes in response to anti-angiogenic therapy.

8934-52, Session 8

Orientation-independent single-shot pulsatile flow measurement using Doppler OCT

Lindsy M. Peterson, Shi Gu, Michael W. Jenkins, Andrew M. Rollins, Case Western Reserve Univ. (United States)

Doppler OCT (DOCT) is capable of providing blood flow velocity information which is critical for investigation of cardiovascular function. However, DOCT is only sensitive to motion parallel with the imaging beam. Methods to obtain absolute flow rates require collecting 3-D volumes to either measure the vessel orientation or to integrate the velocities through an en face cross sectional plane. The en face velocity integration method does not require knowledge of the vessel angle, however to measure pulsatile flow either a gated 4-D DOCT data set must be acquired or the region of interest imaged must remain small. To overcome these limitations we have expanded upon the method of en face velocity integration and applied it to integrating velocities perpendicular to the cross sectional B-scan plane. Velocities perpendicular to the B-scan were acquired using a delay encoded technique to produce two illumination beams with a predetermined angular separation that can image the same point simultaneously. Integrating these velocities from a single B-scan allows for rapid flow measurement without the need for 3-D volumetric data, which enables measurement of pulsatile flow over the duration of multiple heartbeats. To validate this technique, a capillary tube flow phantom was imaged over a range of flow rates provided by a syringe pump. The angle independence of the method was verified by altering the capillary tube orientations. Finally, quail embryo vessels were imaged before and after bifurcations to determine if the combined smaller vessels' flow equaled the original vessel's flow rate.

8934-53, Session 8

Resolving directional ambiguity in light scattering-based transverse motion velocimetry in optical coherence tomography

Brendan Huang, Michael Choma, Yale School of Medicine (United States)

Quantification of flow transverse to the optical axis is important for many problems in medicine. Unlike standard Doppler velocimetry, which is insensitive to transverse flow, the OCT-based dynamic light scattering (DLS) signal is sensitive to transverse motion of scatterers. The basic principle of DLS relies on the insight that although a time-varying OCT signal may be stochastic, owing to the stochastic distribution of scatterers moving through a focal volume, the autocorrelation of that signal has predictable statistics governed by the underlying flow dynamics of those scatterers. DLS-OCT techniques have been recently implemented to measure total transverse speed in scattering media. These methods do not yield directional velocimetry as directional ambiguity arises from a symmetric point spread function (PSF). Here, we propose a novel method of breaking symmetry and resolving directional ambiguity by introducing a variable-speed scan bias along a single axis. By using a novel model of the DLS signal, we successfully isolate a single vector component of total transverse flow speed and thereby unambiguously determine flow directionality. Our method has the advantage of measuring a single transverse velocity component independent of diffusion and orthogonal motion. Notably, our method does not require accurate calibration of the PSF width. We validated our method by quantifying transverse parabolic flow profiles in a capillary tube. We further demonstrated that our transverse velocity measurements are appropriately Doppler angle-dependent. Moreover, the combined transverse DLS signal and axial Doppler signal capture total flow speed.

8934-54, Session 8

Quantitative blood flux measurement using MUSIC

Siavash Yousefi, Ruikang K. Wang, Univ. of Washington (United States)

We propose a super-resolution spectral estimation technique to quantify microvascular hemodynamics using optical microangiography (OMAG) based on optical coherence tomography (OCT). The proposed OMAG technique uses both amplitude and phase information of the OCT signals which makes it sensitive to the axial and transverse flows. The scanning protocol for the proposed method is identical to three-dimensional ultrahigh sensitive OMAG, and is applicable for in vivo measurements. In contrast to the existing capillary flow quantification methods, the proposed method is less sensitive to tissue motion and does not have aliasing problems due fast flow within large blood vessels. This method is analogous to power Doppler in ultrasonography and estimates the number of red blood cells passing through the beam as opposed to the velocity of the particles. The technique is tested both qualitatively and quantitatively by using OMAG to image microcirculation within mouse ear flap in vivo.

8934-55, Session 8

Algorithms for signal and image processing in OCT-based angiography

Ahhyun S. Nam, Isabel Chico-Calero, Benjamin J. Vakoc, Wellman Ctr. for Photomedicine (United States)

OCT-based angiographic imaging relies on signal processing algorithms to generate three-dimensional angiographic datasets, and on image processing algorithms to visualize and quantify these datasets. In each of these areas, we present algorithms that have been tailored to match the properties of OCT signals and OCT angiographic datasets. Further, each algorithm is compared quantitatively against established alternatives. For signal processing, we describe a complex variance algorithm, and compare its performance to alternative approaches including intensity-based Doppler variance and log-domain speckle variance approaches. We demonstrate that the performance of the complex variance algorithm matches or outperforms that of the alternatives. For visualization, we describe optimized algorithms to generate two-dimensional projections from three-dimensional OCT angiographic datasets, and compare to more conventional mean intensity, maximum intensity, and median intensity algorithms. Finally, we describe the automated segmentation of OCT angiographic datasets using advanced geometric model-based segmentation, including robust dataset pre-filtering to remove vascular shadows. At the conclusion, we describe the availability of software resources allowing each of these algorithms to be downloaded and used broadly.

8934-56, Session 8

Localized measurement of longitudinal and transverse flow velocities using optical coherence tomography

Nicolas Weiss, Ton G. van Leeuwen, Academisch Medisch Ctr. (Netherlands); Jeroen Kalkman, Academisch Medisch Ctr. (Netherlands) and Technische Univ. Delft (Netherlands)

Tissue perfusion is a key functional parameter used to describe the supply of blood to tissue. The total supply of blood can be measured in the arteries connected to the tissue. However, the arteries do not give any information about the local delivery of blood. Local delivery of blood takes place in the microvasculature where blood delivers oxygen and removes metabolic waste products. Monitoring of dynamic processes such as diffusion and flow in the microvasculature is a good indicator of the state of tissue perfusion.

Optical coherence tomography (OCT) is an imaging technique in which low coherence interferometry is used to produce depth resolved complex-valued backscatter profiles of (biological) samples up to a few milliliters deep. Several studies have shown the potential of OCT to measure sample dynamics, such as, longitudinal flow and particle diffusion. However, up till now no accurate quantification of the local transverse flow has been achieved for the case of arbitrarily oriented flow.

We present measurements of the path-length resolved OCT signal and its correlation function for the case of arbitrarily oriented flow in the presence of diffusion. Based on our model of the path-length resolved correlation function we obtained accurate results by fitting the model to the measured data with no free/unknown parameters. The model is validated by measuring the transverse and longitudinal flow velocities locally in a colloidal suspension. We show that both sample morphology and flow velocity are determined simultaneously with high spatiotemporal resolution.

8934-57, Session 9

Polarization sensitive optical frequency domain imaging of lung cancer

Lida P. Hariri, David C. Adams, Martin L. Villiger, Brett E. Bouma, Alyssa J. Miller, Matthew B. Applegate, Mari Mino-Kenudson, Melissa J. Suter, Massachusetts General Hospital (United States)

Given the recent emergence of targeted lung cancer therapies, bronchial biopsy and transbronchial fine needle aspiration (TBNA) must yield adequate tumor volumes for both histological and molecular diagnostics. However, the yields with these techniques are often inadequate, in part due to inadvertent biopsy of tumor-associated fibrosis. The ability to assess targeted nodules with optical frequency domain imaging (OFDI) during biopsy is likely to improve diagnostic yield. We have previously demonstrated that OFDI can distinguish lung nodules from parenchyma with high sensitivity and specificity (> 95%). However, structural OFDI cannot differentiate solid tumor from fibrosis. Polarization sensitive OFDI (PS-OFDI) detects birefringence in organized tissues and could be used to distinguish tumor from fibrosis. In this study, PS-OFDI was obtained in 97 lung tumor samples from 32 ex vivo specimens containing varying amounts of tumor and fibrosis. PS-OFDI was obtained with either a custom-built 2.4 Fr (0.8mm diameter) helical scanning catheter or a dual-axis bench top scanner. Strong birefringence was demonstrated in nodules with dense, established fibrosis, with no birefringence in regions of tumor. Tumors admixed with early, loosely-organized collagen showed mild to moderate birefringence. Tumors with little connective tissues showed little to no birefringence. PS-OFDI is capable of differentiate tumor from adjacent fibrosis, and has potential to guide biopsy site selection during bronchoscopy to increase tumor yield.

8934-58, Session 9

Spectral degree of polarization uniformity for depolarization assessment with polarization sensitive optical coherence tomography

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Polarization sensitive optical coherence tomography (PS-OCT) can measure optical tissue properties such as birefringence and depolarization of light. Depolarization has been used to improve tissue discrimination as well as segmentation of pigmented structures. In order to assess depolarization in PS-OCT images, quantities such as the degree of polarization uniformity (DOPU) have been used, which assess the uniformity of polarization states within spatial evaluation kernels. In this presentation, we introduce the spectral degree of polarization uniformity for investigating variations of polarization states between sub-bands of the broadband light source spectrum. We compare the approach to conventional spatial and temporal DOPU algorithms and demonstrate imaging in the healthy and diseased human retina as well as in the pigment epithelium of the rat iris. A spectral analysis of depolarization in PS-OCT images may be of particular interest for investigating processes such as multiple scattering. Analogous to conventional DOPU methods, it may also be used to improve the segmentation of biological tissues and pathologic lesions.

8934-59, Session 9

Characterization of acute and chronic clots in a rat model of deep venous thrombosis with polarization sensitive optical coherence tomography

Martin L. Villiger, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States); Rahmi Oklu, Hassan Albadawi, Harvard Medical School (United States) and Massachusetts General Hospital (United States); William Lo, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States); Michael T. Watkins, Harvard Medical School (United States) and Massachusetts General Hospital (United States); Brett E. Bouma, Wellman Ctr. for Photomedicine (United States) and Harvard Medical School (United States)

The differentiation between acute and chronic thrombus is important because clinical management of deep venous thrombosis (DVT) is dependent on the age of the clot. Administration of thrombolytic agents with an intravenous catheter in combination with anticoagulation therapy is a successful therapy paradigm for patients with DVT, and results in the complete removal of the thrombus, if it is acute (< 2 weeks). Many thrombi are, however, heterogeneous in age, and comprise chronic portions that are composed of collagen and resist this therapy. The thrombolytic therapy should be complemented with mechanical thrombectomy in this case, or the placement of a stent might be required. Catheterization of the culprit vessel is required for either therapeutic measure and would enable diagnostic imaging and informed decision on the appropriate therapy. Here, we investigated the possibility to differentiate between acute and chronic thrombus using polarization sensitive optical coherence tomography (PS-OCT). In a preliminary study we imaged acute and chronic venous clots of a rat model of DVT ex vivo. In addition to the structural intensity signal, we evaluated tissue birefringence, and the degree of polarization (DOP) of spatially averaged Stokes vectors. In chronic clots, the intensity signal experienced less extinction, together with an increased level of birefringence and more pronounced reduction of the DOP along depth than in acute clots. The combination of these several parameters rendered the classification sufficiently robust to be performed through an intravascular PS-OCT catheter, as we demonstrate here with first in vivo measurements of the DVT rat model.

8934-60, Session 9

Monte-Carlo based Bayesian estimator to obtain the true local birefringence of biological samples using polarization-sensitive optical coherence tomography

Deepa K. Kasaragod, Shuichi Makita, Shinichi Fukuda, Simone Beheregaray, Tetsuro Oshika, Yoshiaki Yasuno, Univ. of Tsukuba (Japan)

Recently, Jones matrix optical coherence tomography (JM-OCT) has shown to have the potential towards extending the functional aspect of OCT to understand the microstructural properties from tissue birefringence obtained from phase retardation measurements. However, in JM-OCT system, phase retardation measurements are highly prone to erroneous estimate depending on the signal to noise ratio. So far, a Monte-Carlo based correction method of phase retardation, which is based on a distribution transform of a set of measured phase retardation values and successive mean estimation, has been demonstrated. However, its accuracy is still not sufficient for high-accuracy quantification of tissue birefringence. In this paper, we present a more mathematically sophisticated birefringence estimator based on a Bayesian statistics.

In this paper, we generate Monte-Carlo model to simulate the noise behavior property of local birefringence calculations and obtain a true estimate of the local birefringence using Bayesian approach as a most-likely estimation of true birefringence is obtained from the probability density function. This method has been validated numerically using Monte Carlo simulations. The numerical validation is carried out for 25 Monte Carlo trials for observed local birefringence values obtained for a true local birefringence of 0.002, at an ESNR of 10dB. The Bayesian estimator is also implemented with a 3x3 kernel size across the image in order to estimate the true birefringence over the ESNR distribution. The improved contrast in the local birefringence image obtained for a glaucoma trabeculectomy patient shows the potential of this estimator for the quantification of tissue birefringence.

8934-61, Session 9

Determination of the collagen fiber Brushing direction in articular cartilage by conical-scan polarization-sensitive optical coherence tomography

Zenghai Lu, Deepa K. Kasaragod, Stephen J. Matcher, The Univ. of Sheffield (United Kingdom)

We report on a new articular cartilage imaging technique with potential for in-vivo use that involves supplementing the variable-incidence-angle polarization-sensitive OCT method with a conical beam scan protocol. We show that this new optical technique uniquely can locate the “brushing direction” of collagen fibers in articular cartilage, which is structural information that extends beyond established methods such as split-line photography or birefringent fast-axis measurement in that it is uniquely defined over the full azimuthal-angle range of (-pi, +pi). The mapping of this direction over the cartilage surface may offer insights into the optimal design of tissue-engineering scaffolds for cartilage repair.

8934-62, Session 9

Simplified fiber-based polarization-sensitive swept-source OCT for application to cardiac radiofrequency ablation monitoring

Xiaoyong Fu, Zhao Wang, Yves T. Wang, Michael W. Jenkins, Andrew M. Rollins, Case Western Reserve Univ. (United States)

Radiofrequency ablation (RFA) is the standard of care to cure many cardiac arrhythmias. Currently, there is no technology available for direct visualization of the heart surface during RFA therapy, and RFA treatment is guided by indirect signals. OCT imaging has the potential to meet this need, providing an image of the myocardium at the catheter tip. To provide an image marker of RFA lesion formation that is stable against tissue and catheter motion during imaging in the living, beating heart, polarization-sensitive optical coherence tomography (PSOCT) is expected to be advantageous over conventional OCT. We demonstrate a novel, simple fiber-based PSOCT system to distinguish RFA lesion. A 58.5kHz fourier domain mode locking (FDML) swept source laser with two semiconductor optical amplifiers (SOA) is used to generate 0 and 45 degree linear polarization light for the system, Mueller matrix and Stokes vectors are used to calculation the birefringence information of the sample. The system is demonstrated by imaging human skin in vivo and porcine myocardium with RFA lesions ex vivo. As expected, the untreated myocardium exhibits significant retardance, while the birefringence is clearly abolished in the RFA treated tissue. These results are consistent with our previous experiments and demonstrate the potential of our proposed PSOCT method for monitoring RFA lesion formation. Further optimization and validation are needed before proceeding to in vivo animal studies. Integration of the PSOCT with a catheter probe is underway, and results of catheter-based imaging will be presented.

8934-63, Session 10

New methods for epi-detected self-interference fluorescence microscopy

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We present a new method for high-resolution, three-dimensional fluorescence imaging. In contrast to beam-scanning confocal microscopy, where the laser focus must be scanned both laterally and axially to collect a volume, we obtain depth information without the necessity of depth scanning. In this method, fluorescence is collected in the backward direction and is sent through a phase plate that encodes the depth information into the phase of a spectrally resolved interference pattern. We demonstrate that decoding this phase information allows for depth localization accuracy better than 4 µm over a 500 µm depth-of-field. In a high numerical aperture configuration with a much smaller depth of field, a localization accuracy of 60 nanometers was achieved. This approach is ideally suited for miniature endoscopes, where space limitations at the endoscope tip render depth scanning difficult. We present a new detection scheme based on a detection interferometer which dramatically improves the sensitivity of the technique and opens up possibilities for single-molecule detection.

Early cancer diagnosis can be greatly improved by employing fluorescent labels that selectively target tumors. However, effective endoscopic imaging tools are needed to optimally exploit the potential of these markers. Depth resolved imaging of fluorescence will aid in the determination of the extent of invasion of a tumor in the underlying tissue in real time during intervention. The potential integration with OCT will provide both depth resolved tumor location and information about the surrounding tissue architecture. This could improve the effectiveness of therapy and response monitoring.

8934-64, Session 10

Quantification of cytoarchitecture and myeloarchitecture using optical coherence microscopy

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Label-free volumetric imaging methods can probe brain architectonics without destroying the sample. Recently, microscopic methods like two-photon microscopy, second and third harmonic generation methods, and Coherent Anti-Stokes Raman Scattering (CARS) have been applied to perform cellular level imaging of various structures in the brain. Our lab has developed a dynamic focus-tracked Optical Coherence Microscopy (OCM) technique for deep tissue imaging of the cerebral cortex with intrinsic contrast. In this work, using our OCM technique, we present volumetric imaging and computational techniques to quantify neuronal and myelin architecture with intrinsic scattering contrast both in vivo and ex vivo. In vivo imaging of cytoarchitecture up to depths of about 600 microns reveals the expected cortical laminar cytoarchitecture. Additionally, ex vivo imaging in conjunction with optical clearing techniques enables cellular-level imaging up to the working distance of our objective (3 mm) and clearly shows increased cell density in the middle layers of the cortical column and increased myelin content in the deeper layers of the cortex. We also demonstrate, with application of acetic acid, that source of negative contrast in the images is the entire neuronal cell body and not the nucleus alone. Such clearing and imaging methods, by maintaining tissue morphology, enable non-invasive studies of the brain in situations where exogenous labeling is impractical.

8934-65, Session 10

Enhancement of optical coherence microscopy by using an optical parametric amplifier

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Optical coherence tomography (OCT) is an important approach to ballistic-photon imaging, mainly because of its high sensitivity (near shot-noise limit) and strong coherence gating. However, the detection sensitivity of OCT is limited by the inefficiency and loss in the detection of light signals, and its coherence gate is subject to the inherent trade-off between the suppression of multiply-scattered background and the collection efficiency of ballistic photon signals. Recently, we demonstrated that an optical parametric amplifier (OPA) can be used to enhance the detection of weak light signals in optical imaging in scattering media. The advantages given by the OPA include a high signal gain (more than 30 dB), and the selective amplification of ballistic light based on the inherent time gate and confocal gate that has a nonlinear nature making it unrestricted by the trade-off between the background suppression and signal collection. In this work, we combine these two imaging technologies (OCM and OPA imaging) to investigate the benefit of using an OPA in OCM. It is demonstrated that the OPA enhances the performance of OCM, which is enabled by both the high-level of signal gain provided by the OPA that compensates for the inefficiency and loss in the detection of light signals in OCM, and the nonlinear confocal gate of the OPA that selectively amplifies ballistic light and suppresses the multiply-scattered light background at the same time. This also demonstrates the potential of the OPA to be used as a general-purpose optical amplifier in various optical imaging technologies.

8934-66, Session 10

High-speed OCT / OCM imaging with ultrafast acousto-optic dynamic focusing

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Recent developments of OCT technology provide tools for high-speed imaging with depth ranges achieving few centimeters. Implementation of techniques for depth of focus extension with minimum beam divergence is thus required for long depth range OCT imaging. Standard method involves conical lens (axicon) to generate non-diffracting beams (e.g. Bessel beam). We demonstrate tunable acousto-optic cell generating standing cylindrical ultrasonic wave inside vibrating piezoelectric shell for dynamic focusing. The performance of the acousto-optic device is numerically simulated using successive diffraction model and Fourier optics. This configuration enables modulation of the phase and amplitude of the light beam at the exit plane of the device at the speeds up to 2 MHz. The effects such as ultrafast dynamic focusing and Bessel beam generation due to the controlled wavefront aberrations are confirmed experimentally. Time-averaged light field distribution shows elongated depth of focus. Prototype Fourier-domain OCT and OCM instruments with extended focus are developed. The results of OCT and OCM imaging demonstrate the ability of tunable acousto-optic lens to improve photon collection efficiency and enhance OCT image quality.

8934-67, Session 10

Real-time computed optical interferometric tomography

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High-resolution tomography is of great importance to many area of biomedical imaging, but with it comes several apparent tradeoffs such as a narrowing depth-of-field and increasing optical aberrations. Overcoming these challenges has attracted many hardware and computational solutions. Hardware solutions, though, can become bulky or expensive and computational approaches can require high computing power or large processing times. This study demonstrates memory efficient implementations of interferometric synthetic aperture microscopy (ISAM) and computational adaptive optics (CAO) – two computational approaches for overcoming the depth-of-field limitation and the effect of optical aberrations in optical coherence tomography (OCT). Traditionally requiring lengthy post processing, here we report implementations of ISAM and CAO on a single GPU for real-time in vivo imaging. Real-time, camera-limited ISAM processing enabled reliable acquisition of stable data for in vivo imaging, and CAO processing on the same GPU is shown to quickly correct static aberrations. These algorithmic advances hold the promise for high-resolution volumetric imaging in time-sensitive situations as well as enabling aberration-free cellular-level volumetric tomography.

8934-68, Session 10

Super-resolved reconstruction of optical coherence tomography images by use of multi-penalty conditional random field algorithm

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Spatial resolution enhancement is beneficial for better visualization of fine

details and structures of imaged sample such as observing blood vessels and distinguishing di?erent layers of retina in vascular and retinal OCT images. We propose a Multi-Penalty Conditional Random Field (MPCRF) approach to generate a Super Resolved (SR) OCT image from a set of Low Resolution (LR) OCT images. We add four different penalty factors (spatial proximity, first and second order intensity variations, as well as a spline-based smoothness of fit) in to the the prior model and estimate the final SR OCT image with the help of Maximum A Posteriori (MAP) approach. Applying the proposed MPCRF method on a set of simulated LR OCT images composed from a set of high resolution in-vivo human retinal OCT image, as well as a set of in-vivo rat retinal OCT images, considerably enhances the spatial resolution of the reconstructed image as compared to other tested methods. Visual assessment of the MPCRF results demonstrate the potential of this method in better preservation of fine details and structures of the imaged sample, retaining biological tissue boundaries while reducing the e?ects of speckle noise. This illustrates considerable promise for the proposed MPCRF reconstruction approach as an effective tool to enhance the spatial resolution of OCT images without the need for significant imaging hardware modifications.

8934-69, Session 11

Pre-clinical study design for cancer detection with full-field optical coherence tomography

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The 1um 3D resolution of full-field OCT (FF-OCT) offers views of human tissue that approach histological detail, though the images are captured in a non invasive, non destructive manner without the use of contrast agents. This makes it an attractive tool for use by pathologists to rapidly assess tissue morphology and perform a primary assessment of the pathology. This is of particular interest for quick qualification of biopsied tissue (i.e. verifying that the biopsied tissue has diagnostic value), tumor margin assessment, and in some cases for a primary diagnosis.

Our group has carried out multiple pre-clinical studies to date in a number of organs, such as breast, lymph node, brain, prostate, digestive tract, lung, ear-nose-throat (ENT), in collaboration with radiologists, pathologists and surgeons at several cancer centers. Through these collaborative efforts we have worked to define and perfect the design of these studies in order to standardize the pathologists training on reading the FF-OCT images and measure the learning curve. We have optimized the parameters for matching to histology in order to construct annotated image atlases for each organ.

Here we look at the ensemble of these studies in the context of defining pre-clinical study protocol for validation of a new imaging technology. We present the methodology that has evolved across these studies, and discuss the general and specific issues and solutions that present themselves throughout this process when dealing with medical professionals of different disciplines.

8934-70, Session 11

Longitudinal study of arteriogenesis with swept source optical coherence tomography

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Peripheral arterial disease (PAD) is an atherosclerotic disease of the extremities that leads to high rates of myocardial infarction and stroke, increased mortality, and reduced quality of life. PAD is especially prevalent in diabetic patients, and is commonly modeled by hind limb ischemia in mice to study collateral vessel development and test novel therapies. Current techniques used to assess recovery cannot obtain quantitative, physiological data non-invasively. Here, we have applied hyperspectral imaging and speckle variance OCT to study longitudinal, therapeutically-induced changes in blood oxygenation and vascular morphology, respectively, intravitally in the diabetic mouse hind limb ischemia model. The novel therapeutic treatment system consisted of sustained delivery of fibroblast growth factor 2 (FGF-2) and FGF-9 from “fast” and “slow” release pH- and temperature-responsive microspheres, respectively. The sequential release of FGF-2 and FGF-9 was designed to promote induction of arteriogenesis followed by vessel stabilization and maturation. Quantitative analysis of vascular morphology obtained from Gabor-filtered speckle variance OCT volumes revealed accelerated changes in vascular density and total vessel length in the adductor muscle of an ischemic limb treated with FGF-2/9 in comparison to a blank microsphere-treated limb. Similarly, preliminary hyperspectral imaging data indicated that sustained delivery of FGF-2/9 accelerates recovery of hemoglobin oxygenation distally in the ischemic footpad. The combination of hyperspectral imaging and speckle variance OCT enabled acquisition of novel functional and morphological endpoints from a given mouse, and provides a platform for more robust preclinical evaluations of novel therapies for PAD such as sustained release of FGF.

8934-71, Session 11

Multiparametric, longitudinal OCT in a mouse model of chronic cerebral hypoperfusion

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In vivo optical imaging techniques have recently emerged as important tools for studying neurobiological development and pathophysiology. In particular, two-photon microscopy (TPM) has proved to be a robust and highly flexible method for in vivo imaging in highly scattering tissue. However, two-photon imaging typically requires extrinsic dyes or contrast agents, and imaging depth must be traded off against imaging field-of-view (FOV). Here we demonstrate Optical Coherence Tomography (OCT) methods for longitudinal imaging of hemodynamic and vascular recovery mechanisms in a mouse model of chronic cerebral hypoperfusion. Bilateral carotid artery stenosis was induced using microcoils. An upright 47,000 kHz OCT microscope operating at 1300 nm, using spectral / Fourier domain OCT detection, was built. The axial resolution was 3.6 microns, and the transverse resolution was 7.2 microns. Bilateral thinned-skull, glass coverslip-reinforced cranial windows enabled capillary-scale resolution over weeks without excessively perturbing cortical physiology. Imaging was performed at 8-9 time points per animal over approximately one month. Using a custom-designed tilt stage, the cranium was carefully aligned to the same location across multiple imaging sessions. A Doppler OCT protocol and an OCT angiography protocol were performed in about two minutes each. Using these techniques, cortical flow was quantified in mice longitudinally for time periods of up to one month. In addition, arterial and venous vessel calibers, tortuosity, capillary density, and dynamic red blood cell (dRBC) content were imaged over time. This novel combination of animal model, surgical preparation, and imaging platform enables high-resolution longitudinal imaging studies of mouse models of cerebrovascular disease.

8934-72, Session 11

Evaluation of spontaneous seizure induced neuronal changes using optical coherence microscopy

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Organotypic hippocampal cultures are widely used as a model system to study epileptogenesis in order to develop better treatments for epilepsy. Neuron morphology and number in the brain cultures are used as key parameters to evaluate the status of insult and/or rescue. Here, we present a study utilizing optical coherence microscopy (OCM) for the evaluation of neuronal changes induced by spontaneous seizures in organotypic rat hippocampal cultures. Tissue fixation and staining are not needed for OCM, compared to histology and confocal microscopy typically used to evaluate neuronal injury in the brain cultures. The OCM system was capable of providing axial and transverse resolutions of ~1.5μm and ~2.3μm, respectively. Organotypic hippocampal cultures were prepared from the hippocampus of three 7-day old Sprague-Dawley rats. Three dimensional (3D) OCM images were acquired from different cultures on 7, 14, 21, and 28 days in vitro (DIVs) with a sample size of 9, 11, 9, and 9, respectively. Morphological changes in the hippocampal slices observed with OCM were compared with H&E histology and confocal images to identify features associated with neuronal injury. Viable neurons were identified as hyposcattering circular regions with a well-defined boundary in the OCM images. Statistically significant decreases ($p<0.01$) of total neuron counts in organotypic hippocampal cultures were observed as DIV increased. These results demonstrated that OCM can be used as a promising imaging modality to evaluate neuronal changes in organotypic hippocampal cultures, which opens up new possibilities to investigate treatment mechanism for various neurological conditions such as epilepsy.

8934-73, Session 11

Evaluation of OCT for quantitative in-vivo measurements of changes in neural tissue scattering in longitudinal studies in mouse model of retinal degeneration

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Optical coherence tomography is an imaging modality that is broadly used in ophthalmic diagnostics. The current generation of OCT systems enable reliable acquisition of volumetric scans that permit the extraction of the thicknesses of the different retinal layers, and this comprises the main quantitative analysis available from commercial instruments. Besides monitoring of retinal layers thickness, measurements of OCT signal intensity could also provide information on the scattering properties of the retinal layers. Unfortunately quantitative measurements and interpretation of the changes in retinal OCT is difficult due to variation in overall brightness of the OCT B-scans between imaging sessions. Such variation may be caused by differences in alignment between the OCT system and the imaged eye, changes in focusing, as well as variation in optical quality of the eye optics (changes in the tear film, dilation of pupil etc.). Thus, quantitative analysis of layer intensity requires careful normalization of the OCT data to eliminate potential artifacts. In this manuscript we present the results of evaluating OCT for quantitative changes in OCT signal intensity in longitudinal studies of a mouse model of retinal degeneration. Use of experimental animals enables the performance of well-controlled OCT imaging, including comparison with retina layers intensities at the same retinal locus, acquired in age-matched, wild-type animals. An additional advantage of performing the studies on experimental animals is the possibility of performing histologic evaluation of microscopic changes in retina layers. This study may lead to improved understanding of changes in retinal layer scattering, allowing better interpretation of clinical OCT.

8934-74, Session 11

Morphometric analysis of normal and transgenic murine hearts using optical coherence tomography

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The morphometry of the transgenic murine heart is important in assessing cardiac function and studying cardiac disease. However, due to the small size of the mouse heart, in order to accurately quantify the geometry, a high-resolution imaging system is required. In this report, we demonstrate the use of optical coherence tomography (OCT) in imaging adult murine hearts ex vivo and performing morphometric analyses. The perfusion-fixed heart was first cleared with glycerol to decrease scattering and increase the penetration depth. After clearing, multiple OCT volumes wewre taken at equally spaced rotational increments using a 1060 nm swept-sourced OCT system. A telecentric scan lens was used in the sample arm to minimize non-telecentric scanning. Refraction correction using Snell's law was performed to correct for refraction arising from the top surface of the heart. Next, these volumes are rigidly registered and stitched into a single volume to produce a single OCT volume of the whole heart. From this whole heart OCT volume, the diameters and thicknesses of the various chambers and major blood vessels can be quantified and compared to normal physiological ranges.

8934-75, Session 11

Longitudinal characterization of Drosophila heart development using optical coherence microscopy

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Drosophila heart has been an important model system for investigating cardiac development and differentiation due to its simple organization and similarities with vertebrate heart at early stages of development. Optical coherence microscopy (OCM) that can provide high resolution in both transverse and axial dimensions was utilized in this study for characterizing the Drosophila cardiac function at different stages of its lifecycle. OCM images were obtained at second instar larva (L2), third instar larva (L3), pupa day 1 (PD1), pupa day 2 (PD2), pupa day 3 (PD3), pupa day 4 (PD4) and 7-day old adult stages of wild-type specimens. Heart rate (HR) was one of the parameters that showed significant difference at different developmental stages. HR was highest at the L2 stage, decreased at L3, and further decreased at PD1. The Drosophila heart stopped beating by PD2, restarted beating at PD3, increased at PD4 and returned to an HR close to that of L2 stage in adult flies. For monitoring heart metamorphosis in the pupa stage, 3D and M-mode OCM images of the pupa heart were obtained at an interval of 3 hours until its eclosion. In order to determine functional roles of the Sox102F gene (Drosophila ortholog of human SOX5 gene) in heart development, transgenic Drosophila specimens with RNAi silencing of Sox102F were imaged. Morphological and functional changes of the mutant heart at different development stages were compared to that of age-matched control specimens. Functional and structural parameters showed similar trend in both WT and Sox along the lifecycle, with significant difference observed at some developmental stages.

8934-76, Session 11

Microvascular anastomosis in rodent model evaluated by Fourier domain Doppler optical coherence tomography

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Vascular and microvascular anastomosis are essential to reconstructive microsurgery, vascular surgery and transplant surgery.An imaging modality that provides immediate, real-time in-depth 3D view and flow information of the anastomosis site can be a valuable tool for the surgeon to evaluate surgical outcome. This can be used for both conventional and novel anastomosis techniques, thus potentially increasing the surgical success rate. Microvascular anastomosis for vessels with outer diameter smaller than 1.0 mm is extremely challenging and effective evaluation of the outcome is very difficult if not impossible using computed tomography (CT) angiograms, magnetic resonance (MR) angiograms and ultrasound Doppler.Phase-resolved Doppler optical coherence tomography (OCT) that explores the phase information of OCT signals has been shown to be capable of characterizing dynamic blood flow in clinical settings. In this work, we explore the capability of Fourier domain Doppler OCT as an evaluation tool to detect commonly encountered post-operative complications that will cause surgical failure and to confirm positive result with surgeon's observation. Both suture and cuff based techniques were evaluated on the femoral artery and vein in the rodent model.

8934-77, Session 12

Integrated OCT and OM enables 3D correction of conduction velocity mapping in the early embryonic heart

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Cardiac conduction plays an important role in embryonic heart development. Abnormal conduction patterns and velocities could lead to congenital heart diseases. Optical mapping (OM) is a powerful technique for measuring cardiac electrical activity using voltage-sensitive fluorescent dyes. OM collects information from a 3-dimensional (3D) surface as a 2-dimensional (2D) projection map, ignoring the curvature of the heart surface and imaging angles, resulting in significant errors in conduction velocity measurements. Because looping embryonic hearts are fragile, tiny and more convoluted, conduction velocities of early embryonic hearts have seldom been reported. We demonstrate an integrated OCT/OM imaging system used to calculate conduction velocities in looping embryonic hearts based on surfaces provided by OCT. Image registration ensured accurate alignment of OCT and OM images. A 3D electrical activation map of the looping embryonic hearts is demonstrated for the first time. 3D visualization helps minimize artifacts caused by unusual heart curves in OM. Standard 2D and 3D-corrected conduction velocity calculations were compared at various regions of the heart. A flat region has <5% difference while the outflow tract, with significant curves, shows an average correction of ~25%, which corresponds with the slope estimated from OCT surface rendering. In disease models such as fetal alcohol syndrome, the looping embryonic heart is more convoluted and non-uniformly oriented, making direct comparison of conduction velocity based on projected 2D maps more difficult and error-prone. 3D-corrected conduction velocity calculation thus will provide greater accuracy and enable direct comparisons between normal and abnormal embryos.

8934-78, Session 12

Intracoronary dual-modality optical coherence tomography and near-infrared spectroscopy for coronary artery disease diagnosis

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Coronary artery disease is the result of the buildup and rupture of atherosclerotic plaque within the artery walls. Understanding and diagnosis of these plaques require the knowledge of their microscopic structure as well as their composition. While intracoronary optical coherence tomography (OCT) can acquire microstructural images of the coronary walls in vivo, it is not capable of directly identifying plaque chemical/molecular contents that are associated with their biomechanical instability.

Here we report a dual-modality catheter-based imaging system for simultaneous microstructural and chemical compositional imaging using OCT and diffuse near-infrared spectroscopy (NIRS). Our imaging system, which employs a single high-speed (100 kHz) wavelength-swept light source (1230-1330 nm) for both modalities, uses a dual fiber separated source-detector configuration in a catheter. This device is designed such that it illuminates the tissue through a single-mode fiber and collects the back-scattered light through the same fiber for OCT, while it uses another multimode collection fiber within the catheter, 1-2 mm from the illumination location for NIRS. In the catheter, OCT and NIRS collection fibers are rejoined into a double-clad fiber (DCF) by use of a fiber combiner resident within the catheter. Simultaneous acquisition is enabled by use of a DCF-based rotary junction for cross-sectional imaging and a DCF coupler for NIRS signal extraction/processing. The combined imaging device has the potential to enhance the ability to visualize, identify, and quantify arterial tissue composition. This presentation will describe the design and performance of our OCT-NIRS system and will show our latest results obtained from cadaver human coronary arteries.

8934-79, Session 12

Back-to-back optical coherence tomography-ultrasound probe for co-registered three-dimensional intravascular imaging with real-time display

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We have developed a novel miniature integrated optical coherence tomography (OCT)-intravascular ultrasound (IVUS) probe, with a 1.5 mm-long rigid-part and 0.9 mm outer diameter, for real-time intracoronary imaging of atherosclerotic plaques and guiding of interventional procedures. By placing the OCT ball lens and IVUS transducer back-to-back at the same axial position, this probe can provide automatically co-registered, co-axial OCT-IVUS imaging. To demonstrate its real-time capability, 3D OCT-IVUS imaging of a pig's coronary artery displaying in polar coordinates, as well as images of three major types of atherosclerotic plaques in human cadaver coronary segments, were obtained using this probe and our upgraded system. Histology validation is also presented.

8934-80, Session 12

Multi functional retinal OCT for simultaneous imaging of microvasculature and polarization properties

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We present a multi functional OCT (MF-OCT) system for simultaneous imaging of retinal microvasculature and polarization properties. Both Doppler OCT and polarization sensitive OCT (PS-OCT) are functional extensions of intensity based OCT. Doppler OCT is not only capable of providing 3D microstructural images of the sample but it also permits the visualization of moving particles within tissue. On the other hand, PS-OCT is capable to distinguish between polarization preserving layers, birefringent layers and depolarizing layers. The additional information, provided by PS-OCT, can be used to retrieve quantitative information about a certain retinal layer and to facilitate the segmentation of these layers. Previous combinations of PS-OCT with Doppler OCT were not able to contrast the microvasculature within the human retina. Here we present, to the best of our knowledge for the first time, a MF-OCT system that is capable of visualizing the human retinal microvasculature and tissue polarization properties at the same time. Our MF-OCT system operates at an A-scan rate of 128kHz. 3D volumes spanning over 2.6 x 2.6mm, consisting of 430 x 124 A-scans, were recorded on the retina of a healthy human subject. In order to visualize the microvasculature, 8 consecutive B-scans were recorded at the same lateral position and phase variance B-scans were generated. In addition, averaged intensity B-scans, retardation, optic axis orientation, DOPU and RPE segmentation images were calculated. Afterwards several retinal layers were automatically segmented and en face projections of the retinal capillary network were generated.

8934-81, Session 12

In-vivo mouse model imaging with combined two-photon microscopy and angiographic optical coherence tomography

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Both two-photon microscopy (TPM) and optical coherence tomography (OCT) are 3D tissue imaging techniques with high imaging depths. TPM is based on two-photon excitation, and provides molecular and cellular information of tissues at subcellular resolutions down to a few hundred micrometers deep from the surface [1]. OCT is another 3D technique based on light back scattering, and provides structural information at less than 10 micrometer resolutions down to a few millimeters deep from the surface [2]. TPM and OCT are providing complementary information in their combination system. Various methods to combine TPM and OCT or optical coherence microscopy (OCM) have been developed [3-5]. One method was to use femto-seconds Ti-Sapphire lasers, for a combined TPM and OCM [3]. This method was applied to miniaturized imaging probes with double clad fibers [3, 4]. Another combined TPM and OCT was developed by using two objective lenses of different magnifications for optimizing each method [5]. We recently developed a combined TPM and OCT by using separate light sources for optimal imaging conditions of individual modalities: a wavelength tunable Ti-Sapphire laser and a 1300nm wavelength-swept light source for TPM and OCT respectively [6]. Both TPM and OCT images were obtained with a single objective lens. Local cellular distribution within tissues and tissue structure in the surrounding regions were visualized in 3D. Additional important information of in vivo tissues is vasculature. In tumor microenvironment, abnormal tumor vasculature is one of the hallmarks of cancer. Angiogenesis is required for the growth of tumor and provides route for cancer cell metastasis [7]. There are various OCT methods for vasculature visualization. In this Letter, we developed a combined TPM and angiographic OCT in order to provide vascular information of tissues. Angiographic OCT was implemented by adapting one of OCT vasculature visualization methods. Combined TPM and angiographic OCT was applied to in vivo imaging of mouse models including a cancer model as demonstration.

8934-82, Session 12

A combined OCT- reflectance confocal microscopy approach for real-time assessment of skin lesions

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A combined high-resolution reflectance confocal microscopy (RCM)/ optical coherence tomography (OCT) instrument for assessing skin lesions has been built and preliminarily tested. The initial testing on the skin of volunteers with some burn scars and on ecised skin skinn specimens from cancer patients show that these two optical technologies have complementary capabilities that can offer the clinician a set of clinically comprehensive parameters: OCT helps to visualize deeper injuries and possibly quantify collagen destruction by measuring skin birefringence, while RCM provides submicron details of the integrity of the epidermal layer and identifies the presence of the superficial blood flow. Therefore, the combination of these two technologies within the same instrument may provide a more comprehensive set of parameters that may help clinicians to more objectively and nonivasively assess skin lesion gravity by determining tissue structural integrity and viability.

8934-83, Session 12

High-sensitivity, dual-modality optical coherence tomography and fluorescence needle probe for imaging fluorescently labelled tissue

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Optical coherence tomography (OCT) needle probes offer significant potential for cancer imaging deep in tissue. However, cancer is a complex disease and there are a wide variety of malignant tissue types, many of which have similar optical backscatter properties to some non-cancerous, healthy tissues. This impedes the ability of OCT to detect malignant tissue. Immunostaining of malignant cells using fluorescently labeled antibodies is a technique commonly used in microscopy to identify and differentiate cancer. However, such antibodies may also bind to non-malignant cells and tissue morphology is important to distinguish them. We have developed a high-sensitivity, dual-modality OCT and fluorescence imaging needle probe that can image bound fluorescent antibodies to greatly improve the identification of specific cell types. Our system uses a double-clad fiber coupler to simultaneously acquire and then separate the OCT and fluorescence signals. The focusing optics of the developed needle probe utilize an all-fiber design with improved sensitivity and working distance over earlier work. For the first time, we demonstrate needle imaging of specifically bound antibodies in

human liver tumor samples, validated against images obtained using a standard wide-field fluorescence microscope. We show improved tissue differentiation compared to OCT alone. We believe that dual-modality imaging will substantially improve the diagnostic capabilities of optical needle probes, yielding cell-specific contrast complemented by the morphological information provided by OCT.

8934-84, Session 12

Development of a side-view endoscopic imaging probe for combined two photon microscopy (TPM) and optical coherence tomography (OCT) in mouse colon study

Qingyun Li, Taejun Wang, Ki Hean Kim, Pohang Univ. of Science and Technology (Korea, Republic of)

Colorectal cancer has been a major health problem for human beings, and current detection method based on optical endoscope is not good due to high adenoma missing rate. Colon study based on animal models is useful to test detection and treatment methods. A side-view endoscopic probe for combined imaging of two-photon microscopy (TPM) and OCT was thus developed. The imaging system was realized by adding a grin lens based probe in front of an objective lens, which is a typical part of an imaging system. The probe was 2mm in diameter and 60.79mm in length, with a 90° prism attached at the end deflecting the incoming beam by 90°. TPM used a Ti:Sapphire laser and scanned the sample through scanning mirrors, whereas OCT used a wavelength swept source and scanned through the motion of the sample stage along the probe axis. With the objective lens and the probe in common, TPM and OCT worked sequentially and they switched easily by means of a magnetic mirror. The system was characterized by imaging fluorescent microspheres: for TPM module, approximately 1.5 to 2?m lateral resolution, 15.4 frames per second imaging speed and 250?m in diameter field of view; for OCT module, 13?m axial resolution, 18?m lateral resolution and 1.5mm per second imaging speed. The system was then tested by imaging AOM+DSS mouse model colon samples ex-vivo. OCT visualized microstructures of colon tissues including polyps and TPM showed cellular tubular gland structures and their distortion in polyps.

Conference 8935: Advanced Biomedical and Clinical Diagnostic and Surgical Guidance Systems XII

Sunday - Tuesday 2 –4 February 2014

Part of Proceedings of SPIE Vol. 8935 Advanced Biomedical and Clinical Diagnostic Systems XII

8935-1, Session 1

Autofluorescence Microscopy with sub-300 nm Excitation for Cellular Diagnostics

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Improved methods are needed for evaluating cellular biopsies obtained through needle aspiration or brushings. A novel fluorescence microscope with sub-300 nm excitation has been developed and applied to image cells of human pancreata. The autofluorescence (AF) images are believed the first of their kind in this organ and provide unique visualization of protein content associated with tryptophan. Protein levels were modulated in non-secretory cells with proteasome inhibitor (Velcade), and 40% changes in intensity were observed in the AF images. Very bright AF was observed in primary exocrine cells and shown to originate from protein-rich secretory granules. The technique may be used to probe protein synthesis tied to the unchecked growth of cancer cells and result in a more effective cellular imaging diagnostic. The same instrument was used to visualize blue and green autofluorescence. Blue emission was consistent with signals from mitochondria while green emission was found to originate from lysosomes.

8935-2, Session 1

A compact and portable hyperspectral autofluorescence lifetime point probe system applied to the study of cardiac disease and arthritis

Joao Lagarto, Clifford Talbot, Benjamin T. Dyer, Douglas Kelly, Hugh B. Manning, Imperial College London (United Kingdom); Kazuhiro Yamamoto, Mohammad B. Nickdel, Univ. of Oxford (United Kingdom); Markus B. Sikkel, Imperial College London (United Kingdom); Jayesh Dudhia, The Royal Veterinary College (United Kingdom); Yoshi Itoh, Univ. of Oxford (United Kingdom); Nicholas S. Peters, Alexander Lyon, Christopher Dunsby, Paul French, Imperial College London (United Kingdom)

Fluorescence lifetime measurements can be used to characterize tissue autofluorescence to provide label-free contrast and information concerning tissue metabolism and matrix components without the need for the application of exogenous fluorescent labels and the associated concerns of toxicity and pharmacokinetics. We report the development of a fibre-optic probe-based time-resolved spectrofluorometer utilizing spectrally-resolved time-correlated single photon counting detection and white-light reflectometry. This trolley-mounted instrument is being used to establish clinical efficacy with application to label-free studies of heart disease, investigating the clinical potential of autofluorescence lifetime (AFL) measurements to read out structural and biochemical changes and correlating these with changes in metabolic signals at different stages of disease progression. Preliminary in vivo studies in a rat heart model show statistically significant differences between healthy and failing myocardium. We are also applying this instrument to study the degradation of cartilage in osteoarthritis and have established that there is a decrease in AFL of cartilage specimens that have been enzymatically degraded, e.g. using trypsin, matrix metalloproteinase and bacterial collagenase, to simulate the disease.

The clinical potential of AFL for label-free diagnosis of heart disease, osteoarthritis and cancer would be significantly increased if the cost and size of the instrumentation could be reduced. To develop a low-cost, compact and portable fibre-optic spectrofluorometer that could be easily replicated, we are working on FPGA-based circuitry with an analogue constant fraction discriminator to be used with laser diodes and photomultipliers. Once clinical efficiency and practicality are demonstrated, we believe that such instruments could find wide clinical deployment.

8935-3, Session 1

Fluorescence spectroscopy using indocyanine green for lymph node mapping

Neda Haj-Hosseini, Linköping Univ. (Sweden); Pascal Behm, Fachhochschule NordWestschweiz (Switzerland) and Linköping Univ. (Sweden); Ivan Shabo, Karin Wårdell, Linköping Univ. (Sweden)

The principles of cancer treatment has for years been radical resection of the primary tumor. In the oncologic surgeries where the affected cancer site is close to the lymphatic system, it is as important to detect the draining lymph nodes for metastases (lymph node mapping). As a replacement for conventional radioactive labeling, indocyanine green (ICG) has shown successful results in lymph node mapping; however, most of the ICG fluorescence detection techniques developed are based on camera imaging. In this work, a fiber-optical based spectroscopy system in parallel to an imaging system is developed and evaluated on a tissue-like ICG phantom with ICG concentrations of 6-64 ?M. The system was evaluated intraoperatively on thyroid and additionally on breast. The ICG fluorescence emission and decay characteristics in terms of intensity and peak wavelength showed to be dependant on ICG concentration in the phantom. Fiber-optical based spectroscopy was able to detect ICG fluorescence at significantly lower intensities compared to the camera imaging; therefore, it is expected to improve the detection threshold of the conventional imaging systems when used intraoperatively. The fiber-optical probe allows spectral characterization of the fluorescence and navigation in the tissue as opposed to camera which is limited to the view on the surface of the tissue.

8935-4, Session 1

Early prediction of skin flap viability using visible diffuse feflectance spectroscopy and autofluorescence spectroscopy

Caigang Zhu, Shuo Chen, Nanyang Technological Univ. (Singapore); Christopher Hoe-Kong Chui, Bien-Keem Tan, Singapore General Hospital (Singapore); Quan Liu, Nanyang Technological Univ. (Singapore)

Accurate and early prediction of skin flap viability is vitally important in plastic reconstructive surgery. For the first time, we performed both visible diffuse reflectance and autofluorescence measurements simultaneously on a reverse MacFarlane rat dorsal skin flap model to identify their individual values in the early prediction of skin viability. A total of 62 flap measurement sites from 11 Sprague Dawley rats were

monitored for 72 hours. Fluorescence spectra were used to statistically predict the viability of flaps and quantify physiologically relevant tissue parameters using empirical methods. The results of statistical analysis suggest that either visible diffuse reflectance spectroscopy or autofluorescence spectroscopy alone can predict the viability of flaps with high accuracy; however, autofluorescence spectroscopy is more sensitive to tissue changes in the first two hours after flap elevation. Meanwhile, several physiologically relevant parameters including hemoglobin oxygenation, total hemoglobin concentration and redox ratio indicators estimated from diffuse reflectance and autofluorescence spectra show distinctively different trends over time from non-viable flaps to viable flaps. These findings will be helpful to clinicians for making precise judgment on flap viability.

8935-5, Session 1

High sensitivity intra-operative targeted fluorescence imaging: system, algorithms and early in-human results

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Intra-operative molecular imaging of targeted fluorescence is an emerging field improving surgical and endoscopic results.By visualizing targeted near-infrared fluorescence tracers it is possible to complemented the human visual perception with molecular information even beyond the observed surface in real-time and thus provide the missing information right in the operation room. The concept of micro-dosing is an approach with the potential to accelerate the transfer of fluorescence labeled drugs into the clinics.

The presented intra-operative imaging system is capable to perceive ultra low fluorescence intensities for open surgeries, making micro-dosing in targeted fluorescence imaging feasible.

Early results of a Vascular Endothelial Growth Factor (VEGF)-targeted fluorescence imaging breast cancer study are presented.

8935-6, Session 2

Optical technologies for global women's cancers *(Invited Paper)*

Nirmala Ramanujam, Duke Univ. (United States)

No Abstract Available

8935-8, Session 2

Plasmonic color change assay for detection of nucleic acid disease biomakers

H. H. Wang, Tuan Vo-Dinh, Duke Univ. (United States)

No Abstract Available

8935-7, Session 3

Rapid transdermal bloodless and reagent-free malaria detection

Ekaterina Y. Lukianova-Hleb, Rice Univ. (United States); Kelly M Campbell, Pamela E Constantinou, Janet Braam, John S Olson, Russell E Ware, Rice Univ (United States); David J. Sullivan, Johns Hopkins Bloomberg School of Public Health (United States); Dmitri Lapotko, Rice Univ. (United States)

We report for the first time transdermal, non-invasive, and rapid detection of malaria parasites in blood with high speed, sensitivity and specificity. Detection was achieved with a short near-infrared laser pulse delivered through skin to generate a vapor nanobubble around hemozoin in malaria parasite and transcutaneous acoustic detection of this nanobubble. This mechanism provided the detection in several seconds of as low as three malaria-infected cell per million of erythrocytes in animals in a simple and non-invasive procedure, without using any reagents or drawing blood. Our approach enables the different dimension to diagnose, screen and monitor malaria with a potential to establish a reliable control of this disease among millions of people.

8935-9, Session 3

The study of synchronization of rhythms of microvascular blood flow and oxygen saturation during adaptive changes

Andrey V. Dunaev, Univ. of Dundee (United Kingdom); Victor V. Sidorov, SPE LAZMA Ltd. (Russian Federation); Alexander I. Krupatkin M.D., Central Research Institute of Traumatology and Orthopaedics (Russian Federation); Ilya E. Rafailov, Scott G. Palmer, Sergei G. Sokolovski, Neil Z. Stewart, Edik U. Rafailov, Univ. of Dundee (United Kingdom)

Multi-functional laser non-invasive diagnostic systems, such as "LAKK-M", allow the study of a number of microcirculatory parameters, including blood microcirculatory index (Im) (by laser Doppler flowmetry, LDF) and oxygen saturation (StO2) of skin tissue (by tissue reflectance oximetry, TRO). Such systems could provide significant information for physiology and clinical medicine. The aim of this research was to use such a system to study the synchronization of microvascular blood flow and oxygen saturation rhythms under normal and adaptive change conditions.

Long-term studies were conducted with 8 healthy volunteers – 3 females and 5 males of 21-49 years. A 36 y.o. male volunteer was investigated over 6 months – totalling 100 basic 3 minute tests, including 20 "before and after" tests to monitor effects of exercise. The remaining volunteers were observed over 1-2 months each, totalling over 200 basic tests. Measurements were performed on palmar surface of right middle finger and rhythmic oscillations of LDF- and TRO-graphs were studied using wavelet analysis. Tissue oxygen consumption data (calculated using arterial and venular blood oxygen saturation and nutritive flux volume) for 36 y.o. male during "adaptive changes" (597±109 AU) and after exercise (641±91 AU) demonstrate increased consumption compared to normal (489±131 AU). Data analysis has demonstrated the emergence of resonance and synchronized rhythms of microvascular blood flow and oxygen saturation as an adaptive change in myogenic oscillation (vasomotion) resulting from exercise and potentially from psychoemotional stress. Synchronization of myogenic rhythms during adaptive changes could lead to increased oxygen consumption resulting from increased microvascular blood flow velocity.

8935-10, Session 3

Dermascope assisted interactive patient interface for multiple reference optical coherence tomography

Roshan I. Dsouza, Kai Neuhaus, National Univ. of Ireland, Galway (Ireland); Josh Hogan, Carol Wilson, Compact Imaging, Inc. (United States); Martin Leahy, Hrebesh Subhash, National Univ.

of Ireland, Galway (Ireland)

There has been a growing interest in developing low cost depth-resolved non-invasive dermis imaging tool for both clinical and fundamental investigation of major skin disease. Multiple reference optical coherence tomography (MRO™) is a recently developed miniature time-domain low coherence interferometeric imaging platform, which promises to fit into robust, cost-effective design: virtually solid state, typical of handheld devices. In this paper we demonstrate the feasibility of MRO™ for dermis imaging application by incorporating an derma scope, which provide simultaneous imaging of dermis and an interactive tool for beam steering and registration of the OCT imaging beam at the dermis area. This allows the user to interactively investigate the depth resolved information of any interested target position on the dermis by pointing the mouse pointer within the dermis image. Image acquisition is controlled with software which displays both the dermis and MRO™ axial-scan, and allows detailed information of the depth scan signal to screen for skin disease. We believe this approach will transform an impact on medical care.

8935-11, Session 3

Digital scanning microscope hardware and software: an essential tool for telepathology *(Invited Paper)*

Joe P. Zhou, Chen Liang, DMetrix, Inc. (United States); Rongguang Liang, College of Optical Sciences, The Univ. of Arizona (United States)

The ability to provide pathology service to remote areas of the world and bring patient needs and medical expertise together can have a direct impact on global health. This ability can only be realized only with the use of a digital scanning microscope and its software package that bring together image viewing, sharing, handling, and storage. In this paper, we will examine some basic characteristics of both hardware and software aspects of digital scanning microscope used for telepathology. Additionally, we will also examine some future use of such essential tool.

8935-12, Session 3

Handheld fluorescence lifetime imaging (FLIM) system with real-time image processing

Shuna Cheng, Rodrigo Cuenca, Boang Liu, Bilal H. Malik, Joey Jabbour, Kristen Maitland, Javier Jo, Texas A&M Univ. (United States)

A handheld system for simultaneous multispectral FLIM imagingwith real-time image processing is presented here. The handheld endoscope consists of a 7x13x5 cm3 enclosure with a rigid probe (1.7 cm diameter, 14 cm length). The probe includes a relay lens pair and a third achromat lens working as an objective. The emission is collected through the same three lenses combination and launched to a detection unit outside of the handheld box. In this detection unit, a set of dichoric mirrors and filters is used to separate the emission into three spectral bands, each one coupled into multimode fibers of different lengths to provide optical delay among spectrally resolved fluorescence decays. Thus, for a single excitation pulse, three decays corresponding to three spectral bands are simultaneously detected. In the handheld probe design, lateral resolution and field of view (FOV) can also be trade off by changing of the excitation fiber and the objective in order to tailor the system for a given application.The range for lateral resolution and maximum FOVare tested to be ~35?m to 140?m and ~6.5mm to 13mm, respectively. Real-time image processing was also accomplished by embedding FLIM deconvolution algorithms within the imaging instrumentation. A maximum pixel acquisition and processing of 30 kHz was demonstrated. The system was validated by imaging both fluorescent dyes (NADH, FAD and POPOP) and human oral mucosa in vivo.

8935-13, Session 4

Optically enhanced blood-brain-barrier crossing of plasmonic-active nanoparticles in preclinical brain tumor animal models

Hsiangkuo Yuan, Christy Wilson, Shuqin Li, Andrew M. Fales, Yang Liu, Gerald Grant, Tuan Vo-Dinh, Duke Univ. (United States)

Nanotechnology provides tremendous biomedical opportunities for cancer diagnosis, imaging, and therapy. In contrast to conventional chemotherapeutic agents where their actual target delivery cannot be easily imaged, integrating imaging and therapeutic properties into one enhances the understanding of pharmacokinetic profiles, and enables monitoring the therapeutic process in each individual. Such a concept dubbed “theranostics” potentiates translational research and improves precision medicine. One particular challenging application of theranostics involves imaging and control-delivery of nanoplatforms across blood-brain-barrier (BBB) into brain tissues. Typically, the BBB hinders paracellular flux of drug molecules into brain parenchyma. BBB disrupting agents (e.g. mannitol, focused ultrasound), however, suffer from poor spatial confinement. It has been a challenge to design a nanoplatform not only acts as a contrast agent but also improves the BBB permeation. In this study, we demonstrated the feasibility of plasmonic gold nanoparticles as both high-resolution optical contrast agent and focalized BBB permeation-inducing agent. We specifically examined the microscopic distribution of nanoparticles in tumor brain animal models. We observed that most nanoparticles accumulated at the tumor periphery or perivascular spaces. Nanoparticles were present in both endothelial cells and interstitial matrices. This study also demonstrated a novel photothermal-induced BBB permeation. Fine-tuning the irradiating energy induced gentle disruption of the vascular integrity, causing short-term extravasation of nanomaterials but without hemorrhage. We conclude that our gold nanoparticles are a powerful biocompatible contrast agent capable of inducing focal BBB permeation, and therefore envision a strong potential of plasmonic gold nanoparticle in future brain tumor imaging and therapy.

8935-14, Session 4

The Fluostick: a real handheld system for near-infrared fluorescence image-guided surgery

Paul Dorval, iCube (France) and Fluoptics (France); Norman Mangeret, Stephanie Guillermet, Fluoptics (France); Christian Adrien Righini, Institut Albert Bonniot (France) and Univ. Joseph Fourier (France) and Univ. Hospital of Grenoble (France); Gabriele Barabino, Univ. Jean Monnet Saint-Etienne (France); Philippe Rizo, Commissariat à l’Énergie Atomique (France); Patrick Poulet, iCube (France)

Near-infrared fluorescence image-guided surgery has lately shown a huge potential in oncologic and lymphatic related surgeries. In some indications such as liver, heart or head and neck surgery, fluorescence-reachable anatomic structures are limited by the access to the surgical field. Nevertheless, most of the systems available on the market are too large to image the sides of cavities. Small devices are clearly required to improve workability of fluorescence imaging systems.

The current work describes the development of an instrument and the results of its evaluation. In order to image narrow area, we developed a small size device consisting of an optical head connected to a control box. The whole system, optical head, control box and software, receives

a CE mark for clinical procedures.

Building on existing technologies, we simplified the fluorescence imaging system. It consists of a custom charged-coupled device camera, a high color rendering index visible LED illumination and a Class1 Laser fluorophore excitation. With a curved shape of 25x35x150mm, the optical head was designed as a true handheld probe. The field of view varies from 5x3.75cm to 2x1.5cm. The device is able to collect and display the signal of 0.5pmol of IndoCyanine Green (ICG) with a spatial resolution down to 70μm at 25 frames per second.

The system has been evaluated in pre-clinical and clinical procedures. The preclinical studies confirmed the ability of the system to visualize tumors in mice models. Presented clinical evaluation includes lymphedema investigations and surgical resections of peritoneal carcinomatosis.

8935-15, Session 4

A novel multiwavelength fluorescence image-guided surgery imaging system

Davide Volpi, Iain C. Tullis, Alexander Laios, Univ. of Oxford (United Kingdom); Pubudu N. J. Pathiraja, Krishnayan Haldar, Oxford Univ. Hospitals NHS Trust (United Kingdom); Ahmed A. Ahmed, Borivoj Vojnovic, Univ. of Oxford (United Kingdom)

We will describe development and performance analysis of two clinical near-infrared fluorescence image-guided surgery devices that aim to overcome some of the limitations of current systems. The devices operate in a widefield-imaging mode and can work (1) in conjunction with a laparoscope, during minimally invasive surgery, and (2) as a hand-held, open surgery imaging system. In both cases, narrow-band excitation light, delivered at multiple wavelengths, is efficiently combined with white reflectance light. Light is delivered to ?100 cm2 surgical field at 1-2 mW/cm2 for white light and 3-7 mW/cm2 (depending on wavelength) of red-near infrared excitation, at a typical working distance of 350 mm for the hand-held device and 100 mm for the laparoscope. A single, sensitive, miniaturized color camera collects both fluorescence and white reflectance light. The use of a single imager eliminates image alignment and software overlay complexity. A novel filtering and illumination arrangement allows simultaneous detection of white reflectance and fluorescence emission from multiple dyes in real-time. We will present both fluorescence detection sensitivity modelling and practical performance data.

We have demonstrated the efficiency and the advantages of the devices both pre-clinically and during human surgery. Both the hand-held and the laparoscopic systems have proved to be reliable and beneficial in an ongoing clinical trial involving sentinel lymph node detection in gynaecological cancers. We will show preliminary results using two clinically approved dyes, methylene blue and indocyanine green. We anticipate that this technology can be integrated and routinely used in a larger variety of surgical procedures.

8935-16, Session 4

Real-time three-dimensional fluorescence imaging for surgical guidance

Peng Liu, Junbin Xu, Shiwu Zhang, Pengfei Shao, Univ. of Science and Technology of China (China); Michael F. Tweedle, Ronald X. Xu, The Ohio State Univ. (United States)

Accurate detection of surgical margins and real-time identification of occult diseases are critically important factors contributing to the improved therapeutic outcome. Many intraoperative fluorescence imaging techniques have been developed and experimentally validated. However, most of these imaging techniques are based on 2D projection of fluorescence emission without the three-dimensional (3D) information of tissue structural characteristics. The lack of depth perception in tumor margin detection may provide suboptimal assessment and inaccurate surgical guidance during a tumor resection surgery. To overcome these limitations, we develop a real-time digital fringe projection technique, enabling simultaneous acquisition of fluorescent emission and spatial topography. A digital light processing (DLP) projector is used to produce the structured illumination of three-step shifting fringe patterns on the tumor tissue at a video rate. The patterned reflectance image and the fluorescence emission are captured by a video camera and a near-infrared CCD camera respectively. 3D tumor margin is reconstructed by a real-time shape measurement algorithm that fuses the fluorescent image with 3D tumor topography. The technical feasibility is evaluated by phantom and animal experiments.

8935-17, Session 4

Image-guided plasma therapy for cutaneous wound

Zhiwu Zhang, Wenqi Ren, Univ. of Science and Technology of China (China); Zelin Yu, University of Science and Technology of China (China); Shiwu Zhang, Ting Yue, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

The wound healing process involves the reparative phases of inflammation, proliferation, and remodeling. Interrupting any of these phases may result in chronically unhealed wounds, amputation, or even patient death. Despite the clinical significance in chronical wound management, no effective method have been developed for quantitative image-guided treatment. We integrated a multimodal imaging system with a cold atmospheric plasma probe for image-guided treatment of chronic wound. Multimodal imaging system offers a non-invasive, painless, simultaneous and quantitative assessment of cutaneous wound healing. Cold atmospheric plasma accelerates the wound healing process through many mechanisms including decontamination and coagulation. The therapeutic effect of cold atmospheric plasma is studied in vivo under the guidance of a multimodal imaging system. Cutaneous wounds are created on the dorsal skin of the nude mice. During the healing process, the sample wound is treated by cold atmospheric plasma at a controlled dosage, while the control wound is healed naturally. The multimodal imaging system integrating a hyperspectral imaging module and a laser speckle imaging module is used to collect the information of cutaneous tissue oxygenation and blood perfusion simultaneously, and the edge of the wound site is monitored to assess and guide the plasma therapy. Our preliminary tests show that cold atmospheric plasma in combination with multimodal imaging guidance has the potential to facilitate the healing of cutaneous wounds.

8935-18, Session 4

A goggle navigation system for cancer resection surgery

Junbin Xu, Pengfei Shao, Ting Yue, Shiwu Zhang, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States); Houzhu Ding, Jinkun Wang, University of Science and Technology of China (China)

We describe a portable, low-cost, NIR fluorescence goggle navigation imaging system for image guidance in oncologic surgeries. A 850nm laser diode and a 780nm laser diode are used to provide background illumination and fluorescence excitation in the surgical area. An optical chopper was employed simultaneously to synchronize the background light exposure and NIR fluorescence imaging exposure as well as the camera frame rate. Background light image is captured in real-time, along with the independent channel of NIR fluorescence image by a commercially available miniature CCD camera. An 800nm long-pass filter

is mounted before the camera lens. Grayscale NIR fluorescence images are converted to visible“pseudo-colors”and overlaid onto the background light image. A head mount display (HMD) is used to display the fusion of both the fluorescence image and the background image. Indocyanine Green (ICG) is used as a contrast agent during the imaging process. The performance of the goggle system was quantitatively evaluated on phantoms with different ICG concentrations and on ex vivo tissue models with embedded tumor simulators. Fluorescence images acquired by the goggle system are compared with those of a Xenogen IVIS fluorescence imager. Our preliminary results demonstrate the technical feasibility of using a goggle navigation system to guide the surgical resection of tumors.

8935-19, Session 4

Fluorescence-guided surgical resection of oral cancer

Pierre M. Lane, Catherine F. Poh D.D.S., The BC Cancer Agency Research Ctr. (Canada); J. Scott Durham, Vancouver General Hospital (Canada); Lewei Zhang, Vancouver General Hospital (Canada) and The Univ. of British Columbia (Canada); Miriam Rosin, Calum MacAulay, The BC Cancer Agency Research Ctr. (Canada)

Oral cancer is usually discovered late in its development and is often difficult to treat or remove completely. Data collected at the BC Cancer Agency suggest that the surgical resection of oral lesions guided by the visualization of endogenous tissue fluorescence can dramatically reduce the rate of cancer recurrence. In this talk we will present recent results from this ongoing study.

8935-20, Session 4

Towards femtosecond laser surgery guidance in the posterior eye: utilization of optical coherence tomography and adaptive optics for focus positioning and shaping.

Alexander Krüger, Anja Hansen, Ben Matthias, Tammo Ripken, Laser Zentrum Hannover e.V. (Germany)

Although fs-laser surgery is clinically established in the field of corneal flap cutting for laser in situ keratomileusis, surgery with fs-laser in the posterior part of the eye is impaired by focus degradation due to aberrations. Precise targeting and ensuring of safety distance to the retina also relies on an intraoperative depth resolved imaging. We demonstrate a concept for image guided fs-laser surgery in the vitreous body or modification of the retina itself (with minimized collateral damage) combining adaptive optics (AO) for focus reshaping and optical coherence tomography (OCT) for focus position guidance. The setup of the laboratory system consists of an 800 nm fs-laser which is focused into a simple eye model via a closed loop adaptive optics system with Hartmann-Shack sensor and a deformable mirror to correct for wavefront aberrations. A spectral domain optical coherence tomography system is used to target phantom structures in the eye model. Both systems are set up to share the same scanner and focusing optics. First results concerning the impact of wavefront aberrations and their correction on the threshold of photodisruption in water will be presented and possible solutions to the trade-off between lateral resolution and off-focus intensity drop in the OCT images will be presented. In the near future OCT and AO will be two essential assistive components in possible clinical systems for fs-laser based eye surgery beyond the cornea.

8935-63, Session PSun

Rapid estimation of key tissue parameters from wide-band diffuse reflectance measurements based on sequential weighted Wiener estimation

Shuo Chen, Xiaoqian Lin, Caigang Zhu, Quan Liu, Nanyang Technological Univ. (Singapore)

A new method is presented to noninvasively estimate key tissue parameters, including total hemoglobin concentration (CtHb) and hemoglobin oxygenation (StO2), directly from wide-band measurements. In this study, tissue phantoms with varying CtHb, StO2 and scattering properties are used to mimic human skin and a 3-CCD camera is used to capture the wide-band measurements from the tissue phantoms. The sequential weighted Wiener estimation method is developed to extract the key tissue parameters from wide-band measurements. The results show the excellent agreement between the estimated values and expected values in all parameters. This method opens a new possibility for noninvasive and real-time monitoring of tissue parameters in an imaging setup to investigate the fast changing phenomena.

8935-64, Session PSun

Abnormal heart rate detection device warning via mobile phone network

Adisorn Sirikham, Rajamangala Univ. of Technology (Thailand); Oranicha Jumreornvong, Stanford Univ. (United States); Janta Watcharapong, Rajamangala Univ. of Technology (Thailand)

Heart disease has become one of the top three leading causes of death for over 20 years, with the mortality rate of approximately 85,000 cases per year. Most victims are the elderly. Therefore, the objective of this study is to produce an equipment to assist this group of patients. The equipment consists of 3 major components, including an electrical signal data receiver, a chest strap, and an ECG pole, all of which are attached to the patient’s chest. The data will be sent to a microcontroller to calculate the heart rate. If an abnormal heartbeat is detected, the system will send a warning signal or a short message service (SMS) via a mobile phone network to the patient’s physician or family members. In addition, this device also contains the Global Positioning System (GPS) tracking unit, in which determines the precise location of the patient once the abnormal heartbeat is detected. Therefore, this will allow help to reach the patients experiencing heart beat abnormality. With the use of GSM Module to find the differences between the heart rates measured by the device proposed and by the OMRON SEM-1, a device used in a hospital, this experiment has indicated that the average heart rate measured by the device proposed has fluctuated 2% lesser than the OMRON SEM-1. Moreover, this investigation suggests that the proposed system can detect the abnormal heart rate with 100% accuracy. In addition, the warning signal or the short message service (SMS) can be sent to the physician and relatives punctually.

8935-65, Session PSun

GaAs-based photonic biosensor for detection of E. coli in water

Elnaz Nazemi, Srivatsa Aithal, Walid Hassen, Eric H. Frost, Jan J. Dubowski, Univ. de Sherbrooke (Canada)

Rapid bacteria detection is of a great importance in food safety, water purification and microbial diagnostic applications. We have been developing photonic biosensing (PB) technology based on sensitivity of photoluminescent (PL) emission of III-V quantum semiconductors (QS)

to molecules immobilized on the biofunctionalized surface of GaAs. Since quantum well (QW) PL emission could be made sensitive to the charge accumulation on the semiconductor surface, we investigated the role of this effect on the detection of charged molecules, such as bacteria in water. Accumulation of negatively charged bacteria on the surface of an undoped, or weakly n-doped GaAs QW microstructure could modify the near-surface electric field, as well as reduce the minority carrier surface recombination velocity [1]. Both these effects lead to an increased QW PL intensity. To be useful, however, the biofunctionalized GaAs QW microstructure has to be made relatively stable in a water environment and under laser illumination, while maintaining its sensitivity to target biomolecules. Our QS-PB devices comprise GaAs/AlGaAs heterostructure, and are functionalized with biotinylated anti-E. coli antibody through the link provided by streptavidin to biotin alkanethiol SAM. We have employed ammonium sulphide treatment of biofunctionalized QS-PB biochips and we demonstrate a rapid detection of live E. coli at a detection limit of 10^3 CFU/mL that is improved from the previously reported 10^4 CFU/mL [2].

[1] G. Marshall et al., “Electro-optic investigation of the surface trapping efficiency…”, Nanotechnology 22, 235704 (2011).

[2] V. Duplan et al., “A photoluminescence-based quantum semiconductor …”, Sensors and Actuators B 160, 46-51 (2011).

8935-66, Session PSun

A near-infrared fluorescence-based surgical navigation system imaging software for sentinel lymph node detection

Jinzuo Ye, Chongwei Chi, Shuang Zhang, Xibo Ma, Jie Tian, Institute of Automation (China)

Introduction

Intraoperative near-infrared fluorescence imaging for detection of sentinel lymph node(SLN) in early breast cancer patients was evaluated as a new method. This method can simplify the operation and improve the detection accuracy of SLN detection surgery. In order to perform real-time imaging of SLN detection, we not only need a fluorescence-based surgical navigation hardware system, but also need an easy-to-use imaging software.

Methods

A new surgical navigation system imaging software for SLN detection surgery has just been developed by our research group. This software is designed based on the fluorescence-based surgical navigation hardware system which is developed in our lab, and specifically for intraoperative imaging and postoperative data analysis.

The user interface of the software is designed based on Qt 3.2 and Visual C++ 6.0. The data acquisition module is developed based on PVCAM 2.7 and Pylon2.3 The image processing module is developed based on OpenCV 1.0. Some algorithms have been designed to help achieve the performance of the software, for example, the correlation matching algorithm.

Result

Some of the key features of this software include:

-Set some of the control parameters of the surgical navigation system.

-Acquire, display and automatically store the intraoperative imaging data in real-time.

-Analysis and processing of the saved video and image data.

The developed software has been used to successfully detect the sentinel lymph node (SLN) in breast cancer patients.

Conclusion

In the near future, we plan to improve the software performance and it will be extensively used for clinical purpose.

8935-67, Session PSun

Photooxidization-assisted-photodynamic diagnosis of lymph node metastasis using 5-aminolevulinic acid

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Accurate evaluation of lymph node (LN) metastasis is helpful both for tumor staging and for deciding optimal treatment. In general, pathological diagnosis during surgery, however, takes a long time, and surgery is often delayed until pathological confirmation is obtained. Photodynamic diagnosis (PDD) with 5-aminolevulinic acid (5-ALA) is a useful tool for rapid confirmation of LN metastasis during surgery. Exogenous administration of 5-ALA results in selective accumulation of a fluorescent metabolite, called protoporphyrin IX (PPIX), in cancer cells. PPIX emits red fluorescence at the wavelength of 635 nm. The fluorescence of PPIX is, however, sometimes affected by autofluorescence of tissue chromophores, such as collagen and flavins. In our previous report, we provided a proof-of-principle demonstration of photooxidization-assisted-PDD by using 5-ALA. PPIX changes into another photooxidation-product, called photoprotoporphyrin (PPP), on continuous light excitation. The fluorescence peak of PPP is shifted to the wavelength of 675 nm, while other endogenous chromophores exhibit no spectral peak shift. The analysis of this photoproduct formation allows us to selectively visualize PPIX localization without any effect on the autofluorescence of the tissue chromophores. In this study, we applied photooxidization-assisted-PDD to the estimation of LN metastasis of gastric cancer patients. We obtained regional LN with and without metastasis from gastric cancer patients, and analyzed the spectral images at 635 nm and 675 nm. Although conventional PDD of LN metastasis observing only 635 nm fluorescence sometimes yielded false diagnostic results, we successfully diagnosed LN metastasis of gastric cancer patients by analyzing the photooxidation-product formation.

8935-68, Session PSun

Fluorescence and hyperspectral imaging of sentinel lymph nodes using dyes bounded on superparamagnetic nanoparticules

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Sentinel lymph node (SLN) biopsy is a surgical procedure in which the SLNs are identified, removed, and analyzed to detect a possible metastatic invasion from the primary tumour. A radioactive substance, a dye, or both are usually injected.

Using a probe based on two optical fibers, one for excitation and the other for detection of scattered or fluorescence photons, we measured Patent Blue V (PBV) mixed with human serum albumin (HSA) thresholds lower than 2.5 nmol.L-1. As an extension of this single point detection, SLN mapping can be obtained by fluorescence or by hyperspectral imaging of the distribution of a suitable optical probe.

An experimental setup based on 4 laser diodes, a frontal diffuser and a CCD camera was assembled. Evaluation of the two methods, hyperspectral and fluorescence imaging, for SLN mapping were performed on test objects and on a preclinical model of swollen nodes on Lewis rats.

Images of free dyes (PBV and indocyanin green), superparamagnetic iron oxides nanoparticules (NP), PBV/HSA and PBV/NP complex will be presented. Results are discussed in the perspective of multimodal SLN imaging using dye/NP complex.

8935-69, Session PSun

Optical measurement system for preparation and after OP check of a hip joint endoprothetic implantation

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With a quantity of 290.000 executions the year, the implantation of an artificial hip joint is one of the most common surgery performed in the US. According to prognosis which takes the demographical change into account, the amount of these operations will rise in the forthcoming years.

One of the essential requirements is the perfect reconstruction of the biomechanical conditions, especially the knowledge about the center of the hip rotation and the length of the leg. Based on this information it is possible to ensure the right position of the newly set leg during the surgery.

The goal of this work is to present and evaluate an optical measurement method in order to gather information about the hip joint center and the leg length. An appropriate laboratory setup is designed and implemented in order to evaluate two different approaches. On the one hand a structured light-method consisting of a DLP-Beamer which projects defined patterns on the patient. On the other hand a laser source is used to perform light-section measurements. Additionally both methods are combined with custom software which enables the determination of the hip joint center and the leg length with an accuracy of about 0.2 inches. Both systems operate without any kind of markers.

The clinical usage of the tested approaches would give the surgeon the opportunity to reset the implant-parameters in the course of the surgery. In this way consequence illnesses such as scoliotic pelvis will be prevented.

8935-70, Session PSun

Compact photo-acoustic probe structure for in-vivo clinical image

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In photo-acoustic imaging (PAI) systems, a light illumination pattern is an important parameter because it affects the sensitivity or the maximum image depth. Most PAI systems have an oblique light illumination to an acoustic beam, so they have a standard dark-field illumination and reduced sensitivity. The oblique structure is not compatible to a commercial in-vivo clinical imaging system. In recent, an illumination structure directly to the interest region was introduced for overcoming the limitation, in which the optical connector axis is perpendicular to the ultrasound array axis. This study introduces more compact probe structure compared to the directly illuminated structure, in which the optical connector axis and the ultrasound receiver axis are parallel by using two glass sheets mounted in a water-filled chamber. The glass sheets are tilted 45 degree to the axes.

Laser beam illuminates the interest region through one glass sheet with low transmission loss because the reflective index difference between the glass sheet and water is small. The light-induced acoustic wave in the region propagates back to the sheet, and twice reflects 90 degree off two tilted glass plates. As a result, the acoustic wave is parallel to the receiver and connector axes. The wave propagates with very low reflection loss, since the acoustic wave is totally internally reflected off the 45 degree-tilted glass plates with the critical angle of 19.3 degree in water. The experimental results show the receiving acoustic signal in the proposed probe structure does not have much difference compared with the oblique light illumination structure without any glass plate.

8935-71, Session PSun

New bilirubin meters for neonatal jaundice using laser diodes and LEDs

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Globally, neonatal jaundice is a major cause of newborn death and disability. Neonates with jaundice require monitoring of serum bilirubin which should be repeated at frequent intervals in order to avoid brain damage from inappropriatly high bilirubin levels. An elevated serum bilirubin concentration in the newborn presents a therapeutic as well as a diagnostic problem to the physician. In this paper the authors introduce the theory, design and operating principles of new noinvasive bilirubin meters for transcutaneous bilirubin monitoring in neonatal jaundice . The new bilirubin meters depend upon illuminating the skin of the neonate with radiation from laser diodes and LEDs. The choice of wavelengths follows the principles of optical bilirubinometry. Our new bilirubin meters provide low-cost / high-accuracy simple instruments for either clinical screening or monitoring of serum bilirubin concentration in neonatal jaundice. The new transcutaneous bilirubin meters are useful in countries with heterogenous racial backgrounds like Egypt, and are reliable within a wide range of natural skin pigmentation. Our leading clinical experience as well as the different selection rules of wavelengths will be presented.

8935-72, Session PSun

Nature of autofluorescence in human serum albumin under its native, unfolding and digested forms

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Autofluorescence characteristics of human serum albumin (HSA) are highly sensitive to its local environment. Identification and characterization of the proteins in normal and disease conditions may have great clinical implications. Aim of the present study was to understand how autofluorescence properties of HSA varies with denaturation under urea (3.0M, 6.0M, 9.0M) and guanidine hydrochloride (GnHCl) (2.0M, 4.0M, 6.0M) as well as digestion with trypsin. Towards this, we have recorded the corresponding autofluorescence spectra of HSA at 281nm laser excitation and compared the outcomes. Although, HSA contains 1 tryptophan and 17 tyrosine residues, it has shown intense autofluorescence due to tryptophan compared to the tyrosines in native form, which may be due to the fluorescence resonance energy transfer (FRET) from tyrosine to tryptophan. As the unfolding progresses in denatured and digested forms of the protein, a clear increase in tyrosine fluorescence as compared to tryptophan was observed, which may be due to the increase of tryptophan - tyrosine separation disturbing the FRET between them resulting in differences in the overall autofluorescence properties. The decrease in tryptophan fluorescence of around 17% in urea denatured, 32% in GnHCl denatured and 96% in tryptic digested HSA was observed as compared to its native form. The obtained results show a clear decrease in FRET between tyrosine and tryptophan residues with the progression of unfolding and urea seems to be less efficient than GnHCl in unfolding of HSA. These results

demonstrate the potential of autofluorescence in characterizing proteins in general and HAS in particular.

8935-73, Session PSun

Integrated biosensors in clinical technologies and their intellectual property protection

Dennis Fernandez, Fernandez & Associates, LLP (United States); Antonia Maninang, Stanford Univ. School of Medicine (United States); Shumpei Kobayashi, Univ. of California, San Diego (United States); Gangadharan Sajithlal, Virginia Commonwealth Univ. (United States)

Recent developments resulting from the technological alliance between computer software and biotech have thrown open a wide variety of tools for clinical technologies and systems in the healthcare industry. Diagnosis and clinical data collections are increasingly becoming either personalized or integrated through multi-platforms. A systems biology platform-based network we recently patented integrates biosensors and simulation systems for diagnosis and therapy. This system biologically monitors an individual using biosensors to detect cellular components. Data is simulated or analyzed using systems-biology software, which provides diagnostics or therapeutic guidance. Such tools are revolutionizing clinical technologies and systems in the healthcare industry and the laws of intellectual property rights (IPR) are beginning to keep pace with the changed landscape of technology. The American patent system is currently going through the biggest reformation since the passage of Patent Act of 1952, and thus IPRs and strategies continue to be increasingly vital in these fields. In order to better understand the status quo of intellectual property (IP) specifically in the fields of clinical technologies that apply computational intelligence, basic IP definitions, recent IP developments, and advanced protection strategies are presented and discussed.

8935-74, Session PSun

Passive split ring resonator for continuous physiological sensing through conductivity measurements

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The Split Ring Resonator (SRR) has been developed and explored for a number of sensing technologies and devices. A SRR can be equivalently regarded as an LC circuit; changes in the dielectric environment will change the equivalent capacitance of the resonator, resulting in a shift of the resonant frequency as well as the quality factor (Q-factor). This makes the device a promising application for continuous personal health monitoring throughout the day. In this work, we are developing a passive radio frequency sensor based on ring resonator designs. The targeted frequency band is within 2.4-2.5GHz ISM (Industrial-Scientific-Medical radio band) and is available for medical devices. The resonator structure is first simulated using Finite Difference Time Domain (FDTD) method in CST Microwave Studio to determine the resonant frequency. Then the resonator was fabricated on polyethylene terephthalate (PET) substrate by standard photolithography and lift-off processes. Three biocompatible coatings (PVC, PMMA, and PVDF) were spin-coated on top and tested. Tuning the thickness can further improve the biocompatibility, Q-factor, and resulting sensitivity (mS) of the device. Reflection spectrum (S11) is measured wirelessly using a network analyzer at 100 mW. The current design senses changes in conductivity down to 0.5mS. By reducing coating thickness, reducing the spacing between resonators, and with more efficient resonator designs we expect to further improve this sensitivity. This sensor could be utilized either implanted into the interstitial layer beneath the skin or embedded into a contact lens to sense tear salinity or glucose levels.

8935-75, Session PSun

Assessment of metastatic disease in human lymph nodes via intraoperative optical coherence tomography

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The assessment of lymph nodes is essential for staging cancer since the lymphatic system is one of the principal means for primary tumor cells to metastasize to distal organs. Currently, lymph node status is assessed via sentinel lymph node biopsy in breast cancer patients undergoing lumpectomy or mastectomy procedures. During surgery, the first (or sentinel) lymph node along the axillary lymphatic chain of nodes is excised and sent for time-consuming post-operative histopathological analysis. The majority of these nodes are diagnosed “non-metastatic” and their removal increases patient risk of complications such as lymphedema. Thus, intraoperative assessment of freshly resected lymph nodes could provide immediate feedback for cancer staging, and in situ imaging could potentially reduce the risk of lymphedema by decreasing the number of lymph nodes removed.

We evaluated the efficacy of three-dimensional optical coherence tomography (OCT) for the intraoperative assessment of lymph nodes resected from human subjects during breast and otolaryngology cancer surgeries. We performed a double-blinded study comparing the assessment of the OCT datasets by trained OCT readers, equipped with a decision tree, to post-operative histopathology, the diagnostic gold standard. We evaluated the sensitivity and specificity of the majority vote for each lymph node from our readers’ diagnosis of either “metastatic” or “non-metastatic.” Additionally, we have implemented a handheld OCT surgical probe for assessing in situ and ex vivo lymph node analysis. Our portable 3D-OCT system with micron-scale resolution offers the potential for real-time assessment of lymph nodes in the operating room for time-sensitive surgical evaluation and cancer staging.

8935-76, Session PSun

Heat-induced microbubbles in combination with ultrasound imaging as a potential tool for temperature measurement during thermal ablation surgery

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Background: Thermal ablation therapy offers a minimum invasive way to treat solid tumor and other malignancies. Intra-operative temperature measurement around ablation region is essential to accurately delivery thermal dose to cause necrosis while keep normal tissue nearby unaffected. Commonly used technologies like MRI guided HIFU has several disadvantages including inaccurate temperature measurement or accumulative modeling error and lack sensitivities to adipose tissue.

Methods: Heat-induced microbubbles as contrast agents for clinical ultrasound were fabricated. The dual-shell microbubbles, consisting of poly (lactic-co-glycolic acid) (PLGA) shell and perfluorocarbon core, can respond to thermal stimulus and expand, thus creating hyperechoic

regions which are monitored by clinical ultrasound when delivered to therapeutic region. Microscopic images of microbubble clearly show temperature dependent expansion.

Results: Heat-induced microbubble expansion was demonstrated in simulating phantoms. Microbubbles showed time and temperature dependent size variation under microscopic and ultrasound imaging, which were further characterized statistically. The correlation between microscopic and ultrasound images were also established. In combination with the well-known Arrhenius model for assessment of hyperthermia treatment which describes the relationship between time dependent temperature and cell death, the microbubbles offers a potential way to measure temperature related necrosis around thermal ablation region intra-operatively using clinical ultrasound.

Conclusion: Heat-induced microbubbles in combination with clinical ultrasound show the concepts of a cost-effective yet accurate way for cell death measurement during thermal ablation therapy. This idea shows the possibility of using clinical ultrasound for temperature measurement of thermal ablation surgery.

8935-77, Session PSun

Automation of a dispersive Raman spectrometer using LabVIEW aiming in vivo diagnosis of skin cancer

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The development of optical techniques for minimally or non-invasive diagnosis of human tumors, and consequent discrimination between malignant, benign and normal tissues, can lead to rapid tumor diagnosis in situ with high sensitivity and specificity. Integrated optical systems that perform automated collection of spectra and provide diagnostic information in vivo in real time is a challenge for modern medicine. This work aimed to automate the spectra collection of a dispersive Raman spectrometer for use in in vivo experiments of skin cancer diagnosis. The routine of data collection, storage, pre-processing and processing of spectral data was developed within the Scientific Imaging Toolkit (Roper Scientific) under the computational environment LabVIEW (National Instruments), which has virtual instruments to control the excitation parameters (laser power), spectrometer (exposure time, number of accumulations), pre-processing signals (cosmic rays filtering, removal of fluorescence background and normalization) and spectral analysis (spectral models based on PCA and tissue biochemistry) in real time. The “RamanLife” routine allowed the use of the Raman spectrometer in procedures for skin tumors removal, with the estimation of the relative amount of basal compounds that constitute the biological tissues, such as lipids, phospholipids, proteins, amino acids, nucleic acids among others, is obtained during the collection of spectra. This provided reliable diagnostic information useful to differentiate between neoplastic, benign and normal tissues, offering the possibility of routine use in clinical diagnosis and in supporting histopathological evaluation for neoplastic skin lesions in vivo in real-time, avoiding unnecessary incisional biopsies and thus enabling assessments in large population groups.

8935-78, Session PSun

Continuous noninvasive in vivo monitoring of intravascular plasma volume and hematocrit changes in response to blood removal and fluid replacement in a rat model

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We report a new algorithm and measurement system that permits simultaneous monitoring of the hematocrit and plasma volume fraction of blood within the intravascular space of an optically probed volume of skin. The system involves probing with a near infrared laser and simultaneously collecting the Rayleigh and Mie scattered light as one raw signal and the undifferentiated Raman and fluorescence emission as the second raw signal. Those two physically independent raw signals and six parameters that can be obtained by either direct calculation or empirical calibration permit monitoring of the blood in rat paws. We tested a device based on the algorithm in the context of improving detection of blood loss for people with an early undiagnosed internal hemorrhage via real-time monitoring of signal changes with direct correlation to hematocrit. We performed IACUC allowed experiments monitoring rat paw skin in vivo while removing blood, centrally or peripherally, and then adding replacement fluids such as Normocarb and blood. Blood removal itself elicits a consistent response, decreasing hematocrit and increasing relative plasma volume, that depends on the rate and location of removal, the total amount of blood removed, the location of monitoring, and possibly other factors as yet unknown. Similarly, replacing the blood with whole blood vs. saline consistently produces a rational range of responses. Calibration across subjects and the measurement of absolute hematocrit will also be discussed.

8935-79, Session PSun

Multispectral tissue analysis and classification towards enabling automated robotic surgery

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Accurate optical characterization of different tissue types is an important tool for potentially guiding surgeons and enabling automated robotic surgery. Multispectral imaging and analysis have been used in the literature to detect spectral variations in tissue reflectance that may be visible to the naked eye. Using this technique, hidden structures can be visualized and analyzed for effective tissue classification. Here, we investigated the feasibility of automated tissue classification using multispectral tissue analysis. Broadband reflectance spectra (200-1050 nm) were collected from nine different ex vivo porcine tissues types using an optical fiber-probe based spectrometer system. We created a mathematical model to train and distinguish different tissue types based upon analysis of the observed spectra using total principal component regression (TPCR). Compared to other reported methods, our technique is computationally inexpensive and suitable for real-time implementation. Each of the 92 spectra was cross-referenced against the nine tissue types. Preliminary results show a mean detection rate of 91.3%, with detection rates of 100% and 70.0% (inner and outer kidney), 100% and 100% (inner and outer liver), 100% (outer stomach), and 90.9%, 100%, 70.0%, 85.7% (four different inner stomach areas, respectively). We conclude that automated tissue differentiation using our multispectral

tissue analysis method is feasible in multiple ex vivo tissue specimens. Although measurements were performed using ex vivo tissues, these results suggest that real-time, in vivo tissue identification during surgery may be possible.

8935-80, Session PSun

Photon-tissue interaction model for quantitative assessment of biological tissues

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In this study, we describe a direct fit photon-tissue interaction (PTI) model to analyze human tissue optical reflectance spectra quantitatively. Improvements to previous PTI models include rapid, real-time analysis of spectra (reduction from > 1 minute for previous PTI analysis to < 1 second for direct fit PTI analysis) and improved model fit accuracy with an optimized least-squares fitting procedure. Biologically relevant optical tissue parameters extracted with the direct fit PTI model include parameters associated with cellular nuclear features, such as diameter and refractive index, and absorber concentrations (e.g., oxygenated and deoxygenated hemoglobin). This direct fit PTI model was tested on measured reflectance spectra from excised human pancreatic tissues. The parameters extracted from diseased pancreatic tissues were significantly different than those from normal pancreas, a finding consistent with histopathological assessments and our previous human studies. These results suggest that reflectance spectroscopy coupled with the direct fit PTI model potentially can be tools to rapidly and quantitatively assess biological tissues for human disease detection.

8935-21, Session 5

Shifted excitation Raman difference spectroscopy using a dual-wavelength DBR diode laser at 671 nm

Martin Maiwald, Jörg Fricke, Arnim Ginolas, Johannes Pohl, Bernd Sumpf, Götz Erbert, Günther Tränkle, Ferdinand-Braun-Institut (Germany)

In recent years, the market of compact portable Raman sensor systems such as handheld devices has grown rapidly and led to in situ analysis in various application fields. Food safety, detection of explosives, and point of care diagnostic are only a few examples where Raman spectroscopy goes out of the laboratory. However, disturbing sources such as fluorescence, ambient light, and a fixed pattern noise interfere on the Raman signals and complicate the analysis of unknown substances. Here, shifted excitation Raman difference spectroscopy (SERDS) has proven to be a promising technique to separate Raman signals from a disturbing background.

In this contribution the application of SERDS using a dual-wavelength Y-branch distributed Bragg reflection (DBR) diode laser at 671 nm will be presented.

Firstly, the design of the excitation light source will be presented. The device has a footprint of 0.5 mm x 3 mm and consists of two integrated laser cavities. Deeply etched surface DBR gratings were designed for a spectral spacing suitable for SERDS. A Y-branch coupler section is implemented to realize one output aperture and thus provide one excitation spot on the sample.

Secondly, electro-optical and spectral properties will be discussed with respect to the requirements for SERDS. An optical power of 110 mW is achieved at a heatsink temperature of 25°C in cw-operation mode for both wavelengths at 670.5 nm and 671.0 nm.

Finally, Raman experiments are carried out using selected samples and different measurement scenarios to demonstrate the suitability of the dual-wavelength diode laser for SERDS.

8935-22, Session 5

In vivo Raman spectroscopy as a clinical tool to detect biochemical change in the pregnant cervix

Christine M. O'Brien, Elizabeth Vargis, Jeffrey Reese, Kelly Bennett, Anita Mahadevan-Jansen, Vanderbilt Univ. (United States)

Preterm birth (PTB) is the leading cause of infant mortality, and occurs in 1 in 8 pregnancies worldwide. There is no single sensitive tool available that allows clinicians to identify women at high risk for PTB. Many risk factors for PTB exist; however, over half of all patients with PTB do not have any risk factors. A tool that could accurately provide early detection of labor onset and etiology of preterm labor would be invaluable for improved patient outcomes. Prolonging delivery by even 24-48 hours allows for successful administration of corticosteroids, providing monumental improvement in lung development when given enough time to take effect. We propose using in vivo Raman spectroscopy to measure biochemical change in the human cervix over the course of pregnancy as a means to evaluate the state of the cervix and predict risk of preterm birth. Our group has previously detected drastic biochemical changes using in vivo Raman spectroscopy in pregnant mice. In this study, data from 40 pregnant human subjects will be collected during prenatal visits over the course of pregnancy. Multivariate methods including creation of generalized linear models will provide intra- and inter-subject comparisons to evaluate how spectra change longitudinally. Spectral features indicative of labor onset will be determined, and the underlying biochemical components responsible for such deviations will be identified.

8935-23, Session 5

In vivo sensing of plasmonic nanoprobes using surface-enhanced raman

Scattering detection

Tuan Vo-Dinh, Janna K. Register, Andrew M. Fales, Hsin-Neng Wang, Eugenia H. Cho, Alina Boico, Duke Univ. (United States); Natalie A. Wisniewski, PROFUSA, Inc. (United States); Thies Schroeder, Duke Univ. (United States); Bruce Klitzman, Duke Univ. School of Medicine (United States)

This presentation describes the development and applications of plasmonics-active surface-enhanced Raman scattering (SERS) nanoprobes for in vivo sensing in animal studies. Plasmonics refers to the research area of enhanced electromagnetic properties of metallic nanostructures that produce ultrasensitive and selective detection technologies. Plasmonics-active nanosystems are at the cutting edge of biomedical 'theranostics' (combination of both therapeutics and diagnostics) and show exciting promise for clinical translation to early disease detection. The plasmon resonance of anisotropic noble metal nanoparticles can be tuned to coincide with the "tissue optical window", allowing very efficient sensing and imaging of nanoparticles with low absorption from surrounding biological media. These nanoparticles can be modified on the surface with biocompatible coatings in order to increase in vivo stability as well as to provide for targeting and other detection mechanisms. Raman spectroscopy affords the capability of reporting rich molecular information about multiple contrast agents

simultaneously. Combined with noble metal nanoparticles, the SERS effect increases the Raman signal by several orders of magnitude-making plasmonics-active SERS nanoplatforms an important new tool for the medicine of the future. In this presentation, we will present a nanostar-based SERS probe, a unique nano-platform for in vivo detection. Our group has developed a simple, surfactant-free method for synthesizing gold nanostars that does not require the use of toxic chemicals. We will present our NIR portable Raman setup and in vivo SERS detection results in both small animal (rats) and large animal models (pigs) to illustrate the potential of SERS nanoprobe platforms for in vivo biosensing.

8935-24, Session 5

Resonance Raman spectroscopic characterization of oral cancer blood plasma

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Raman Spectroscopy is a molecular vibrational spectroscopy technique used to collect and examine the uniqueness of the chemical fingerprint of the molecules under investigation. When the metabolic end product of the cells goes into the blood circulation, the components and contents of the biological molecules and their local environment will be changed. Based on this, a study was carried out to discriminate oral cancer patients (9 samples) from that of normal subjects (9 samples), utilizing the resonance Raman technique. The blood plasma was excited by using Argon laser of wavelength 488 nm and Raman spectra were acquired for each blood plasma using Lab RAM HR instrument. From the obtained spectra it was observed that the Raman bands of cancer blood plasma corresponding to ?-carotene occurs at 1158 cm-1 (C-C stretch vibration) and 1527 cm-1 (C=C stretch vibration) are red shifted compared to that of normal subjects. The intensity ratio of resonance Raman band C=C stretch vibration to that C-C stretch vibration found to be less for cancer blood plasma to that of normal blood plasma. Further, the Raman spectra have been analyzed employing the multivariate statistical methods - principal component analysis (PCA) and linear discriminant analysis (LDA). An accuracy of 88.9% was obtained using Linear Discriminant Analysis.

8935-25, Session 5

Device for 3 dimensional, real time and intraoperative evaluation of surgical margin status

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It has been shown that the presence of cancer cells within the 2mm margin of resected breast tumors is strongly correlated with the risk of local tumor recurrence. Margins are thus directly correlated to the success of breast cancer surgeries. Standard histopathology provides a definitive diagnosis of margin status, but results may take several days. If the margin contains tumor cells, patients have to undergo a second surgery to remove more tissue. Consequently, there is a need for rapid, accurate, automated guidance tool that can be used during tumor resection to assure complete removal in a single procedure.

We have demonstrated that spatially offset Raman spectroscopy technique can be used to evaluate the margin of breast tissue with 95% accuracy. However, the technique is based on single point measurement. To cover the entire tissue surface of the specimen, sequential measurements are required.

In this talk, we will present a 3D scanning device that can measure the entire surface of a resected tumor. The device can automatically reconstruct the 3D image of a tumor, scan its entire surface and evaluate its surgical status in real-time. Tests have been carried out on risk-reducing mastectomy samples and shown good match with histopathology results. With this device, intra-operative guidance can thus be achieved. This instrument would be able to diagnose the margin status of a excised tumor while patient is still in the OR so that if more tissue needs to be removed, it can be done immediately rather than requiring a second surgery.

8935-26, Session 6

Reflectance confocal microscopy of oral epithelial tissue using an electrically tunable lens

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Confocal microscopy is a powerful technique which allows for high resolution imaging of intact tissue by rejecting out of focus light to generate optical sections of the sample. Most of the current embodiments of confocal microscopy utilize a translation stage to scan the sample in the axial direction. In this paper, we present the use of a commercially available electrically tunable lens (ETL) to achieve axial scanning in a reflectance confocal microscope (RCM). We characterized the RCM in order to demonstrate and account for the changes in the optical performance of the system as a function of focal length of the ETL. The field of view at the sample was measured to be 625 µm in diameter, and over an axial range of 275 µm, the lateral and axial resolutions varied from 1 to 2 µm and 4 to 14 µm, respectively, both dependent on the variable focal length of the ETL. Finally, RCM imaging was performed on normal human biopsies from the oral cavity ex vivo. Sub-cellular morphologic features were seen throughout the depth of the epithelium while axially scanning using the focus tunable lens. The results indicate that although the range of ETL's focal length can limit the full depth scan potential of a confocal microscope, it is still adequate to image biological tissue with relatively thin regions of interest such as in the oral epithelium.

8935-27, Session 6

Mueller polarimetry for the detection of cancers

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Polarization measurements allow one to enhance imaging contrast of superficial tissues and obtain new polarization sensitive parameters for better description of the micro- and macro- structural and optical properties of complex tissues. Since the majority of cancers originate in the epithelial layer, probing the morphological and pathological changes in the superficial tissues using an expended parameter set and with improved spatial resolution and contrasts will give new clues on the early clinical detection of cancers. For such purposes, various polarization imaging techniques have been reported in recent years, including Mueller matrix imaging which allows the most comprehensive characterization of the samples. In fact, all parameters of the existing polarization techniques can be expressed as functions of the Mueller

matrix elements. In recent studies we carry out Mueller matrix imaging on various types of cancerous tissues and look for cancer specific features. Using a scattering model, which approximates the anisotropic biological tissues to a mixture of spherical and cylindrical scatterers imbedded in birefringent ambient media, and a Monte Carlo simulation program, we examine in detail the relationship between the micro structure of the model represented by the parameters of the spheres, cylinders and the ambient medium, and the characteristic features of the Mueller matrix. The studies help to understand the contrast mechanism of polarization sensitive measurements for different cancers and provide the basis for potential clinical applications.

8935-28, Session 6

An optimized dual photoelastic modulator polarimetry system for the investigation of biological tissues, with applications to breast cancer

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Optical polarization properties of a sample are best extracted from its Mueller matrix, a complete mathematical description of a sample's interaction with polarized light. A major difficulty with polarization studies of biological tissues is that their turbid nature causes light to undergo multiple scattering events, resulting in increased depolarization. Therefore, it is imperative to detect and isolate the weak, information-carrying, remaining polarized fraction with a high level of accuracy and high signal-to-noise ratio. The dual photoelastic modulator (dual PEM) polarimetry system combines dynamic polarization modulation with synchronous detection to address this challenge. However, the choice of input polarization states with which to probe the sample is arbitrarily left to the experimenter. Previous work in our lab has theoretically demonstrated that there is an optimum set of input polarizations to use when examining a sample with a dual PEM system. Here we provide experimental validation that the sample Mueller matrix is most robustly determined, with a dual PEM system, when the sample is probed with a specific set of input polarizations whose Stokes vectors form a cube on the Poincare sphere. This optimized dual PEM polarimetry platform is now being used to perform cancer detection, and treatment monitoring studies in excised mice mammary tissues. We will compare these findings to the results of complimentary non-linear microscopy investigations of collagen structure by collaborators at the University of Wisconsin to gain a more complete biophysical picture of the complex breast cancer milieu, with potential for an optical polarimetry platform for breast cancer diagnosis.

8935-29, Session 6

Endoscopic 3x3 and 4x4 Mueller matrix polarimetric tissue imaging system

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Mueller polarimetric imaging is a promising technique for surgical imaging to provide additional (structural and compositional) information. However, it is challenging to miniaturise polarisation state generator (PSG) and polarisation state analyser (PSA) required by Mueller matrix measurements. Previously, a 3?3 Mueller polarimetric endoscopic system was constructed by rotating a non-birefringent endoscope with a linear polarizing film covering on its illumination channel and analysing polarisation states at the eyepiece to avoid PSA miniaturisation. In this work, the same endoscope and PSA were employed together with a rotatable endoscope sheath with a ring-shaped linear polarizing film covering on (non-contact) double crescent shaped endoscope illumination channel. This overcame previous problems caused by the change of field of view during the rotation of the whole endoscope. 4?4 Mueller polarimetric imaging was also conducted based on a rotating-retarder-fixed-linear-polarizer method (RR-FP) by using the same sheath with a ring-shaped quarter-waveplate at the distal end as a RR, and a linear polarizing film fixed on the illumination channel as a FP. FP was adjusted to be horizontal and four optimized angles of the RR were selected as -45°, 0°, 30° and 60°.

3?3 Mueller images of rat abdomen were obtained and decomposed to quantify depolarization, diattenuation and linear retardance. The depolarization power (range 0-1) of different organs/tissue with 546 nm illumination showed significant differences (kidney 0.2, liver 0.2-0.3, small bowel and stomach 0.6, renal cyst 0.8, fat 0.9). In retardance image, the fat was heterogeneous and the stomach was contrasted with other organs. All the observed organs exhibited very weak diattenuation. 4?4 Mueller polarimetric imaging was validated with a depolarizer and a rotating linear polarizer on top of paper and showed a good fit to theory. Our work shows that 3?3 Mueller imaging is promising in tissue diagnosis and that it is feasible to implement 4?4 Mueller imaging endoscopically.

8935-30, Session 7

IgG/anti-IgG immunoassay based on a turn-around point long period grating

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Long period gratings have been proposed as label-free optical biosensor for a few years. Refractive index changes, which modify the transmission spectrum of the fiber, are still used for evaluating a biochemical interaction that occurs along the grating region. This is a substantial option with respect to other label-free optical approaches, such as surface plasmon resonance, interferometric configurations and resonating structures. A turn-around point (TAP) long period grating was manufactured by etching the fiber cladding for pushing the refractive index sensitivity near to its theoretical maximum of these devices. Considering the simplicity and the fast process with respect to the silanization procedure, the functionalization of the fiber was carried out by Eudragit L100 copolymer. An IgG/anti-IgG immunoassay was implemented for studying the antigen/antibody interaction. A comparison of the biosensor performance was made between the TAP long period grating and a standard one manufactured without any etching process. Experimental results demonstrated an enhancement of the biosensor performance when a TAP long period grating is used with respect to a standard long period grating. A dynamic signal range of more than 6 nm and a limit of detection lower than 100 ?g L-1 were achieved, thus providing another step forward in the field of optical biosensors based on fiber gratings.

8935-31, Session 7

The Application of Surgical Navigation System Using Optical Molecular Imaging Technology in Orthotopic Breast Cancer and Metastasis Studies

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We used optical molecular imaging technology to help surgeons to observe orthotopic tumors and metastasis for intraoperative resection and visualize tumor border for precise positioning. The luciferase expressing breast cancer cell line 4T1-luc was used for in vivo analysis. The tumor-bearing nude mice were given tail vein injection of MMP 750FAST probe. Hematoxylin and eosin staining confirmed that there was metastasis with the fluorescence tissues.

8935-32, Session 7

The optics inside an automated single molecule array analyzer

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Quanterix and Stratec Biomedical have developed an instrument that enables the automated measurement of multiple proteins at concentration ~1000 times lower than existing immunoassays. The instrument is based on Quanterix's proprietary Single Molecule Array technology (Simoa TM) that facilitates the detection and quantification of biomarkers previously difficult to measure, thus opening up new applications in life science research and in-vitro diagnostics. Simoa is based on trapping individual beads in arrays of femtoliter-sized wells that, when imaged with sufficient resolution, allows for counting of single molecules associated with each bead. This is also known as digital ELISA.

The platform developed is a merger of many science and engineering disciplines. This paper concentrates on the optical developments that have enabled the advancement of this technology. At the core of the system is a custom, wide field of view, fluorescence microscope that images the array to detect single molecules and beads. The consumable disc containing 24 microstructure arrays was developed in close collaboration with Sony DADC. The system cadence requirements, array dimensions, and requirement to detect single molecules led towards some challenging optical requirements. Specifically the wide field of view resulted in the need for a custom objective lens. In addition, cost considerations for the Analyzer platform required a custom solution that also took advantage of image processing capabilities so to take some of the burden from the optical requirements. This paper will discuss the design considerations and resultant optical architecture that has enabled the development of an automated digital ELISA platform.

8935-33, Session 7

Fluorescence imaging for intraoperative detection of the parathyroid glands in endocrine surgery

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When surgical resection of diseased tissue is the only curative option, patient outcome is strongly dependent on accurate identification of surgical margins. In endocrine procedures, accuracy of the surgeon's visual inspection is required to avoid inadvertent removal or trauma to the parathyroid gland, the gland responsible for calcium regulation. Feasibility of parathyroid detection has been shown using fluorescence spectroscopy because the parathyroid emits a near-infrared (NIR) autofluorescence signal 2-11 times higher than the thyroid. While spectroscopy provides point-based information, we aim to further the technology by creating a NIR fluorescence imaging system to provide the surgeon with 2D spatial information. To determine the optimal system for parathyroid imaging, we developed and compared three systems: a NIR viewer adapted from a photomultiplier tube, a cooled CCD camera, and an adapted clinical endoscope camera. These systems were characterized on the basis of linearity, field-of-view, spatial resolution, contrast, feature retention, and ex vivo imaging.

Results show the ability of all systems to image brighter autofluorescence emitted from the parathyroid than the thyroid. However, a tradeoff exists between performance and ease-of-use. The cooled CCD camera exhibits the highest contrast, but the bulk and cost of this system reduces its appeal for clinical settings. The endoscope camera is the most easily adaptable in the operating room but shows non-linearity in the power range of interest. Results indicate the NIR viewer is the optimal system for intraoperative parathyroid imaging because it is a low-cost, portable system that provides enough contrast and resolution for anatomical guidance during surgery.

8935-34, Session 7

Background fluorescence reduction and absorption correction for fluorescence reflectance imaging

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Intraoperative fluorescence imaging in reflectance geometry (FRI) is an attractive imaging modality as it allows to noninvasively monitor the fluorescence targeted tumors located below the tissue surface. Some drawbacks of this technique are the background fluorescence decreasing the contrast and absorption heterogeneities leading to misinterpretations concerning fluorescence concentrations.

We presented a FRI technique relying on a laser line scanning instead of a uniform illumination. Here, we propose a correction technique based on this illumination scheme. We scan the medium with the laser line and acquire at each position of the line both fluorescence and excitation images. We then use the finding that there is a linear relationship between the excitation intensity profile and the background fluorescence one. This allows us to predict the amount of signal to subtract to the fluorescence images to get a better contrast. As the light absorption information is contained both in fluorescence and excitation images, this method also permits us to correct the effects of absorption heterogeneities, leading to a better accuracy for the detection.

This technique has been validated on simulations (with a Monte Carlo code and with the diffusion approximation using Nirfast) and experimentally with tissue-like liquid phantoms with different levels of background fluorescence. Fluorescent inclusions are observed in several configurations at depths ranging from 1 mm to 1 cm. Results obtained with this technique are compared to those obtained with a more classical wide-field detection scheme for the contrast enhancement and to the fluorescence to excitation ratio approach for the absorption correction.

8935-35, Session 8

Multispectral imaging of organ viability during uterine transplantation surgery

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Uterine transplantation surgery has been proposed as a treatment for permanent absolute uterine factor infertility (AUFI) in the case of loss of the uterus. Due to the complexity of the vasculature correct reanastomosis of the blood supply during transplantation surgery is a crucial step to ensure reperfusion and viability of the organ.

While techniques such as fluorescent dye imaging have been proposed to visualise perfusion there is no gold standard for intraoperative visualisation of tissue oxygenation. In this paper results from a liquid crystal tuneable filter (LCTF)-based multispectral imaging (MSI) laparoscope are described. The system was used to monitor uterine oxygen saturation (SaO2) before and after transplantation. Results from surgeries on two animal models (rabbits and sheep) are presented.

A feature-based registration algorithm was used to correct for misalignment induced by breathing or peristalsis in the tissues of interest prior to analysis. An absorption spectrum was calculated at each spatial pixel location using reflectance data from a reference standard, and the relative contributions from oxy- and deoxyhaemoglobin were calculated using a least squares regression algorithm with non-negativity constraints.

Results acquired during 10 surgeries show that cornual oxygenation changes are consistent with those observed in point measurements taken using a pulse oximeter, showing reduced SaO2 following reanastomosis. Values obtained using the MSI laparoscope were lower than those taken with the pulse oximeter, which may be due to the latter's use of the pulsatile arterial blood signal. Future work incorporating immunological test results will help to correlate SaO2 levels with surgical outcomes.

8935-36, Session 8

Multispectral digital colposcopy (MDC) for detection of clinical cervical intraepithelial neoplasia

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We continue to develop a MDC device for visualization of the entire cervix using a high-resolution camera (3Mpixel) to collect a fluorescence RGB images using a number of excitation wavelengths (350+20 nm, 420+20 nm, 460+10 nm) and reflectance RGB images using cross-polarized (broad-spectrum white, 420 nm and 460 nm) illumination. We intend to use this MDC in conjunction with an attached spectroscopic point probe (reflectance broad-spectrum white: 350+20 nm, 420+20 nm, 460+10 nm fluorescence), where the former is applied as a high sensitivity wide field of view screening approach with the latter applied to refine biopsy site selection.

8935-37, Session 8

Wide-field high-resolution imager for optical pathology

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To enable automated and registered wide-field and high-resolution optical pathology we have developed a system that combines digital CCD macro-imaging with multimodal confocal imaging. It enables viewing biotissue using several optical modalities, including reflectance, fluorescence and fluorescence polarization, side-by-side and on different scales. The system provided wide-field images of 3 cm x 4cm with a lateral resolution of 12 ?m and an estimated axial resolution of 50 ?m as well as high-resolution images with a field of view of 250 ?m, an axial resolution of 3-5 ?m, and a lateral resolution of better than 0.9 ?m. To evaluate the performance of the system we have imaged fresh excess human skin, breast and brain tissues, obtained from surgeries. The tissues were stained in Demeclocycline (DMN) and imaged. Reflectance and fluorescence signals were excited with a 402 nm diode laser and fluorescence emission of DMN was measured between 455 nm and 495 nm. Imaged specimens were processed for H&E histopathology and compared to the optical images. Our results show the feasibility of using the developed device and methods for rapid and accurate optical evaluation of pathology.

8935-38, Session 8

Diffuse and fluorescence optical tomography with a fully integrated time-gated near-infrared spectroscopic imaging device

Patrick Poulet, Virginie Zint, Renee Chabrier, Wilfried Uhring, Univ. de Strasbourg (France)

A time-resolved, spectroscopic, diffuse and fluorescence optical tomography device was assembled for clinical applications like brain functional imaging and intraoperative fluorescence imaging. The entire instrument lies in a unique setup that includes the light sources, an ultrafast time-gated intensified camera and all the electronic control units. Four near infrared laser diodes are driven by a nanosecond electrical pulse generator working at a repetition rate of 100 MHz. The light pulses, less than 80 ps FWHM, are injected in a four-furcated optical fiber ended with a frontal light distributor to obtain a uniform illumination spot. Back-scattered or fluorescence photons are selected by suitable filters mounted on a wheel and detected by the intensified CCD camera. There are resolved according to their time of flight. The photocathode is powered by an ultrafast generator, at 100 MHz, a width corresponding to a 200 ps gate, with a 10ps minimum delay increment. The photocathode and pulse intensifier have been specially designed for this application. The whole instrument is controlled by an FPGA based module. All the acquisition parameters are configurable via software through an USB plug and the image data are transferred to a PC via an Ethernet link. The compactness of the device makes it a perfect device for bedside clinical and surgical applications. The instrument will be described and characterized. Preliminary data recorded will be presented.

8935-39, Session 8

Implementation and evaluation of Google Glass for visualizing real-time image and patient data in the primary care office

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Primary care physicians (PCPs) must conduct a staggering number of comprehensive physical examinations and medical record reviews in a timely yet accurate manner, resulting in demanding daily schedules. Few commercial technologies have been marketed towards primary care due to limited profit margins, which has subsequently hampered improvements in disease screening and detection, work flow, records management, and interaction between physician and patient. With a recent rise in the use of consumer electronics (e.g. smart phones and

home monitoring) for point-of-care diagnostics, more efficient and modernized strategies can be adopted.

PCPs have very limited access to new diagnostic screening tools. By integrating advanced optical imaging technology with a traditional otoscope, we have introduced an influx of new information to PCPs. This also requires the data to be effectively managed and displayed for useful real-time evaluation. We have integrated Google Glass©, a commercial heads-up display (HUD), into our portable primary care imaging system that integrates optical coherence tomography (OCT) and video imaging within a MEMS-based handheld scanner. The HUD is linked with the computer and imaging system, allowing patient medical record data to be displayed before the physical exam, as well as real-time OCT and video image data during the exam. Use of this HUD enables the physician to focus on the patient, rather than the display, during an exam. The implementation of this consumer HUD in primary care imaging is expected to facilitate the collection, visualization, and use of image-based data for disease screening and early detection in primary care medicine.

8935-40, Session 8

Using tissue phantoms to determine the relationship between blood vessel depth and size from thermal images

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Previously we developed a thermal imaging technique that uses heat as a contrast agent to image vascular structures in tissue. This study describes the development of an algorithm to determine the depth and size of vessels in tissue from thermal images for 3-D mapping of the vasculature. We are applying this imaging technique to the detection of breast cancer as the imaging of blood vessels provides a direct probe of tumor growth and its potential for metastasis.

We used tissue phantoms and heated wires to simulate warmed blood vessels in soft tissue. Phantoms were created using gellan gum with similar thermal properties as soft tissue. Resistive wires with diameters of 0.5 to 2.0 mm were embedded within the phantoms at depths of 0 to 4.0 mm. The phantoms were heated to body temperature and the wires were heated 0 to 10.0 °C above body temperature. Thermal images of the phantom surface were taken for all wire sizes, depths and temperatures. The profiles and derivatives of temperature across the wire were analyzed.

The temperature profiles of the wires were Gaussian-like and broaden as wire depth increased for a given diameter. The absolute value of the spatial derivative yielded two Gaussian-like peaks. It was found that the distance between these two peaks increased as a function of wire depth. The wing of the profile varied as a function of wire diameter. Based on these results, an algorithm was developed to determine wire depth and size from IR images.

8935-41, Session 9

Full-field optical coherence tomography for rapid qualification of deep organ microbiopsies

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Endoscopic ultrasonography EUS-guided fine-needle-aspiration-biopsy (FNAB) has come to serve an important role in preoperative/pretherapeutic characterization and triage of deep organ lesions, allowing definitive cell/tissue diagnosis. FNAB are acquired via the digestive tract using <1mm diameter needle, producing fine strings of cell/tissue specimens <1mm thick and up to 50mm long. Although rapid on-site evaluation (ROSE) of the material is possible in some centers, it requires presence of a cytotechnologist/pathologist in the operating room, which can be impractical for outpatients. In these conditions, delayed assessment can reveal that this material is not representative of the lesion, containing only digestive tract contaminant or blood clots. A pilot study was performed to evaluate the feasibility of using full-field OCT (FFOCT) for immediate specimen quality assessment.

35 samples were imaged from 15 patients, of esophageal, gastric, pancreatic, hepatic, and lymph-node formalin-fixed FNAB. FFOCT images (1µm 3D resolution) were acquired and later compared with paraffin cell block of the same sample. We were able to identify blood, mucus, muscle, collagen, digestive mucosa, and recognize abnormal architectural features that disturb tissue morphology (eg infiltrative pancreatic ductal carcinoma). However we were not able to detect lesions at the individual cell scale (eg small cell lymphoma).

FF-OCT offers fast, non-invasive, non-destructive imaging that can be inserted into the pathology lab workflow to provide rapid differential diagnosis. In future, this assessment could be performed in the operating room during the EUS-FNAB procedure by the endosonographer while the patient is present, to approve satisfactory specimens and reduce patient recall.

8935-42, Session 9

Motion tracking to enable pre-surgical margin mapping of basal cell carcinoma using optical imaging modalities: initial feasibility study using optical coherence tomography

Megan Duffy, Thomas Richardson, Emma Craythorne, Raj Mallipeddi, Andrew J. Coleman, Guy's and St Thomas' NHS Foundation Trust (United Kingdom)

A system has been developed to assess the feasibility of using motion tracking to enable pre-surgical margin mapping of basal cell carcinoma (BCC) in the clinic using optical coherence tomography (OCT). This system consists of a commercial OCT imaging system (the VivoSight 1500, MDL Ltd., Orpington, UK), which has been adapted to incorporate a webcam and a single-sensor electromagnetic positional tracking module (the Flock of Birds, Ascension Technology Corp, Vermont, USA). A supporting software interface has also been developed which allows positional data to be captured and projected onto a 2D dermoscopic image in real-time.Initial results using a stationary test phantom are encouraging, with maximum errors in the projected map in the order of 1-2mm. Initial clinical results were poor due to motion artefact, despite attempts to stabilise the patient. However, the authors present several suggested modifications that are expected to reduce the effects of motion artefact and improve the overall accuracy and clinical usability of the system.

8935-43, Session 9

Improved method to visualize lipid distribution within arterial vessel walls by 1.7 µm spectroscopic spectral-domain optical coherence tomography

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We report an improved method to visualize lipid distribution in axial and

lateral direction within arterial vessel walls by spectroscopic spectral-domain Optical Coherence Tomography (OCT) at 1.7μm wavelength for identification of lipid-rich plaque that is suspected to cause coronary events. In our original method, an extended InGaAs-based line camera detects an OCT interferometric spectrum from 1607 to 1766 nm, which is then divided into seven subbands, and A-scan OCT profile is calculated for each subband, resulting in a tomographic spectrum. The tomographic spectrum is decomposed into lipid spectrum having an attenuation peak at 1730 nm and non-lipid spectrum independent of wavelength, and the weight of each spectrum is calculated as score. In this paper, we present an improved algorithm, in which we combine the lipid score and the non-lipid score to derive a corrected lipid score, and the way of combination is optimized so that sensitivity and specificity in lipid detection is maximized. We have found that the corrected lipid score is better than the raw lipid score in that the former is more robust against false positive occurring due to abrupt change in reflectivity at vessel surface. In addition, we have optimized spatial smoothing filter so that false positive and negative due to detection noise and speckle is minimized. We have verified the improved algorithm by measuring porcine carotid artery and lard by our 1.7μm OCT system (n = 4 samples), and confirmed that the sensitivity and the specificity of lard is improved from 73% to 94%.

8935-44, Session 9

Linear source spectral-domain OCT for diagnosis of ocular and skin diseases

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Spectral-domain Optical coherence tomography (SD-OCT) is an established clinical imaging tool for diagnosis of eye diseases. Sensitivity of the current SD-OCT technology is not able to provide enough penetration depth, so that many tissue structures associated with glaucoma, myopia and other diseases cannot be clearly visualized. The current OCT sensitivity also limits the imaging speed, so that the image quality is often degraded by artifacts caused by eye motion. In this work, we developed a Linear-source SD-OCT technology, and demonstrated advantages of the proposed technology over the existing SD-OCT technology in penetration depth.

The proposed technology can be used to enhance SD-OCT sensitivity by more than one order of magnitude. This advance in sensitivity can produce increased penetration depth with imaging acquisition rate equal to the current technology. Further clinical application of the proposed technology may enable visualization of important tissue structures associated with above mentioned diseases which cannot be visualized clearly using the current SD-OCT technology.

8935-45, Session 9

Validation of a bronchoscopic anatomical optical coherence tomography system for quantitative airway geometry

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An anatomical optical coherence tomography (aOCT) system was developed toward the goal of providing accurate surface geometries in the upper airways of newborns with subglottic stenosis. The system consists of a long coherence length 1305nm wavelength-swept light source operating at 5 kHz. It provides 25 um axial resolution and a beam waist of 384 um at a maximum depth range of 12 mm, which is comparable to the maximum size of the upper airway in children under 10 years old. A distally scanned fiber-optic ball lens catheter of 820 um outer diameter was inserted into the side port of a small-bore flexible bronchoscope for helical scans. With digital dispersion compensation, the operational SNR was 90.2 dB. To quantify the accuracy of the system, aOCT images of tubes up to 25 mm diameter were segmented, and the deviation from true geometry were quantified. At a rotational rate of 10 Hz and translation speed of 2.5 mm/s the measured tube diameter was accurate within 55 um, with a point-to-point standard deviation of 110 um. Then, aOCT scans were acquired of a 3D-printed opaque plastic airway phantom reconstructed from CT data of a 10 year old normal boy. 3D visualizations of the phantom were in agreement with the known phantom geometry. Finally, an ex vivo porcine airway was imaged from the carina to the top of the trachea during a mock procedure using the small-bore bronchoscope. The capability to accurately reconstruct airway geometry with this system may enable predictive modeling of airway obstructions.

8935-46, Session 10

Combined NIR absorption spectroscopy and OCT for neurovascular bundle proximity sensing during dental implant surgery

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We present proof of concept work towards an in situ neurovascular bundle proximity sensor using combined near-infrared (NIR) absorption spectroscopy and optical coherence tomography (OCT). Many surgical procedures rely on computer-assisted navigation techniques built from preoperative imaging. The use of these static models introduces errors in localization of key anatomy during surgery. An in situ sensor could warn surgeons of the proximity of vital tissue structures. For example, dental implant procedures pose a risk of damage to the inferior alveolar nerve (IAN) in the mandible. It is standard procedure to use preoperative x-ray and/or CT scans to guide implants to a depth of approximately 2mm from the perceived location of the IAN based on the expected error of using preoperative images. However, implant viability is dependent on osseointegration, which improves with increased implant depth. A probe that could sense proximity to the IAN and/or its neurovascular bundle could allow dental surgeons to both place implants deeper for better implant viability, as well as reduce the chance nerve damage. We integrated an in-house detector card customized for low frequencies to demonstrate proof of principle for low resolution NIR absorption spectroscopy sensitivity to the pulsation of the artery in the IAN neurovascular bundle in the 0.5-3mm range in a custom phantom model. OCT provides finer resolution in the 0-0.9mm range. These two techniques combined could provide a complete solution for proximity sensing of the IAN bundle during dental implant procedures. Preliminary in vitro and progress towards in vivo experiments are discussed.

8935-47, Session 10

Optical coherence tomography-aided anastomosis platform study in the rodent model

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Anastomosis is one of the most commonly performed procedures in the surgical field that involves tubular structures, such as blood vessel, lymphatic vessel, seminal duct and ureter. Suture based anastomosis is still the foundation for most basic surgical training and clinical operation, although advanced none-suture based anastomosis techniques are being

developed. For tubular structure anastomosis, immediate real-time post-operative evaluation of the surgical outcome is critical to the success of surgery. Previously evaluation is based on surgeons' experience and ultrasound imaging. Fourier-domain optical coherence tomography is a high-speed, high-resolution noninvasive 3D imaging modality that has been widely used in the biomedical research and clinical study. In this study we assess the Fourier-domain optical coherence tomography based anastomosis evaluation of lymphatic vessels, seminal duct, ureter and oviduct in the rodent model. Immediate post-operative and long term surgical site data were collected and correlation study with histology analysis was also performed. Critical clinical parameters such as lumen patency, anastomosed site narrowing and suture error detection will be provided to surgeons. An OCT-aided anastomosis platform is the long-term study goal.

8935-48, Session 10

In vivo intra-operative breast tumor margin detection using a portable OCT system with a handheld surgical imaging probe

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Breast-conserving surgery is a frequent option for women with stage I and II breast cancer, and with radiation treatment, can be as effective as a mastectomy. However, adequate margin detection remains a challenge, and too often additional surgeries are required. Optical coherence tomography (OCT) provides a potential method for real-time, high-resolution imaging of breast tissue during surgery. Intra-operative OCT imaging of excised breast tissues has been previously demonstrated by several groups. In this study, a novel handheld surgical probe-based OCT system is introduced, which was used by the surgeon to image in vivo, within the tumor cavity, and immediately following tumor removal in order to detect the presence of any remaining cancer. Following resection, the researchers in this study imaged the excised tissue with the same probe for comparison. We present OCT images obtained from over 15 patients during lumpectomy and mastectomy surgeries. Images were compared to post-operative histopathology for diagnosis. OCT images with micron-scale resolution show areas of heterogeneity and disorganized features indicative of malignancy, compared to more uniform regions of normal tissue. Video-rate acquisition shows the inside of the tumor cavity as the surgeon sweeps the probe along the walls of the surgical cavity. This demonstrates the potential of OCT for real-time assessment of surgical tumor margins and for reducing the unacceptably high re-operation rate for breast cancer patients.

8935-49, Session 10

Demonstration of 3D imaging of skeletal muscle at centimeter depths using a 30-gauge side-viewing optical coherence tomography needle probe

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Evaluation of muscle structure is important in the assessment of a range of diseases, such as Duchenne muscular dystrophy. Histological analysis is the gold standard, but is undesirably invasive in diseased muscle. Optical coherence tomography (OCT) has been shown capable of imaging muscle structure but has been restricted to only imaging superficial tissue.

We have developed an extremely miniaturized OCT needle probe, in which the distal focusing optics are encased within a 30-gauge needle (outer diameter 310μm). The focusing optics comprise spliced lengths of no-core and gradient-index fiber, terminated with angle-polished, gold-coated no-core fiber to redirect the beam perpendicular to the probe. By optimizing the optical design and manufacturing process for the OCT needle probe, we were able to gain significant improvements in sensitivity over the previously published design for such small probes. Interfaced to a 1310nm swept source OCT, the probe achieved a sensitivity of 108dB.

Using ex vivo samples from mouse models, we assessed the ability of the OCT needle probe to image muscle structure at centimeter depths within both healthy muscle tissue, and dystrophic tissue with regions of necrosis. Acquiring multiple 3D image volumes, the probe allowed the visualization of individual myofibers, appearing as striations in the OCT images. In necrotic regions within the dystrophic muscles, the degradation of cellular structure was seen as a loss of these striations. Structures such as fascicles, tendon and connective tissue were also visualized. These findings demonstrate the potential of OCT needle probes to image disease-related changes in muscle.

8935-50, Session 11

Spectral encoding enhances visual flexibility of surgical endoscopes

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Endoscopic cameras are a key component for endoscopic/robotic-assisted surgical procedures.

Unfortunately, endoscopes lack a human peripheral visual field, making it challenging for the operator to gain situational awareness of the scene. This is a typical trade-off in imaging systems: for a given numerical aperture, the field of view cannot be increased without sacrificing resolution. However, the spectral content of the illumination provides another degree of freedom, as different wavelenghts can encode information. We present a spectral multiplexing technique for endoscopes to provide peripheral vision while minimally affecting the forward image quality of existing systems.

Our system uses multiplexing filters, oriented at 45 degrees relative to the optical axis, before and after a rigid boroscope (Olympus). One spectral channel is reflected by both filters and imaged onto one camera, and the second channel is transmitted by both filters and imaged onto a second camera. The separation filter is a multilayer dielectric filter. For our proof-of-concept experiment, we used dichroic filters designed for 567 nm at 450 (Thorlabs DMLP567). The current black & white images can be provided as full true color high quality images using custom designed multiband filters similar to ones used for separation of images in the Dolby 3D cinema standard.

We believe our multiband-channel technique enables additional flexibility for surgical endoscope systems. Future implementations of this system

may include an actuator for the filter at endoscope tip to provide a full 360 degree circle of peripheral views. Additionally, the illumination strength can be tuned independently for both channels.

8935-51, Session 11

System for clinical photometric stereo endoscopy

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In many endoscopic applications, tissue topology is a critically important factor in accurately assessing lesions. However, conventional endoscopy captures only color and intensity contrast of the field of view, and consequently, tissue topology can only be inferred through indirect cues. To capture more information about the object shape, our laboratory has recently introduced Photometric Stereo Endoscopy (PSE): a technique that obtains information about the high-spatial-frequency topology of the field of view simultaneously with the conventional color image. In this work, we describe the principles of PSE, progress on our development of a PSE system for human clinical testing, and various solutions to visualize contrast obtained with PSE.

Using a benchtop PSE prototype, we demonstrate PSE imaging of adenomas in ex vivo human gastrointestinal tissue. Our clinical PSE system consists of a commercial gastroscope, a set of four optical fibers, and an alignment cap. The custom pieces are biocompatible and can be sterilized before assembly in the endoscopy suite. The resulting endoscope has the same outer diameter as a conventional colonoscope (14 mm), plugs in to a commercial video processor, captures PSE images at 15 Hz, and displays a conventional color movie to the gastroenterologist. We show that this system can capture topological contrast of 1 mm tall bumps at working distances that are typical of colonoscopy screening. This system will enable PSE to be clinically evaluated as a tool for providing topological contrast in endoscopy.

8935-52, Session 11

Evaluation of the three-dimensional endoscope system for assessing the gastrointestinal motility

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This paper described evaluation of the three-dimensional endoscope system for assessing the gastrointestinal motility. Gastrointestinal diseases are mainly based on the morphological or anatomical abnormity. However, sometimes the gastrointestinal symptoms are apparent without visible abnormalities. Such diseases are called functional gastrointestinal disorder, for example, functional dyspepsia, and irritable bowel syndrome. One of the major factors of these diseases is the gastrointestinal dysmotility. Assessment procedures for motor function are either invasive, or indirect. We thus propose a three-dimensional endoscope system for assessing the gastrointestinal motility. To assess the dynamic motility of the stomach, three-dimensional endoscopic imaging of stomach lining is performed. Propagating contraction waves are detected by subtracting estimated stomach geometry without contraction waves from one with contraction waves. After detecting constriction waves, their frequency, amplitude, and speed of propagation can be calculated. In this study, we evaluate the proposed system. First, we evaluate the developed three-dimensional endoscope system by a flat plane. This system can measure the geometry of the flat plane with an error of less than 10 percent of the distance between endoscope tip and the object. Then we confirm the validity of a prototype system by a wave simulated model. The detected wave is approximated by a Gaussian function. In the experiment, the amplitude and position of the wave can be measure with 1 mm accuracy. These results suggest that the proposed system can measure the speed and amplitude of contraction. In the future, we evaluate the proposed system in vivo experiments.

8935-53, Session 11

A catheter-based fluorescence tomography platform

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Optical molecular imaging has limited depth penetration mainly due to highly scattering nature of the tissue. However, it has a great potential for non-invasive cancer imaging using specific organ dedicated platforms including catheter-based applications. Fluorescence imaging has its advantages over absorption based imaging since its sensitivity is superior and can detect smaller amount of contrast agents. Extensive efforts have recently been spent to develop molecular optical fluorescent probes that preferably target cancerous tissue. We are developing a catheter based fluorescent tomography system. It will be consisting of two side-shooting optical fibers moving independently, one to illuminate the tissue with excitation light and the other for collecting the emitted light from near-infrared (NIR) fluorescent molecular probes. The excitation and emission in the NIR range of the spectrum will allow locating these agents deep in tissue due to low tissue absorption in this range. A mechanical assembly using computer controlled actuators will enable the user to move or rotate the optical fiber bundle in the transparent catheter with high precision. It will have predefined linear and angular positions to provide fast and easy-to-use interface for the operator during the imaging procedure. Performance of the imaging system is currently being evaluated with extensive phantom studies using fluorescent inclusions of various concentrations and sizes located at different depths. These studies will allow us to characterize the limits of the system in detecting the lesions with respect to their size, dept, accumulated targeted probe concentration. The next step will be testing this platform in vivo.

8935-54, Session 11

Development of a forward-looking rigid imaging probe for wide-field fluorescence and optical coherence tomography

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Development of imaging probes is important to access internal or external organs with optical imaging technologies since optical imaging techniques have limitation in the imaging depth. We developed a forward-looking rigid imaging probe for simultaneous wide-field reflectance/fluorescence and optical coherence tomography. The combination of wide-field fluorescence and OCT is useful in clinical study, because fluorescence imaging is an effective way to get molecular specificity and OCT imaging provides information of undersurface tissue

microstructures. The size of distal end of probe was a 12 mm in diameter and 180 mm in length, and FOV were approximately 6 mm. For wide-field fluorescence imaging, illumination beam was delivered via optical fiber bundle surrounding densely the outer surface of imaging probe. The OCT resolutions were measured by point spread function (PSF) through imaging the microsphere, and each were 15 μm in the lateral and 10 μm in the axial direction, and depth of focus (DOF) was 1.6 mm. Acquisition time was approximately 1.89 seconds per 3-D volume. The probe was attached to a simple articulated arm for flexible positioning. Its performance was tested with a fluorescent microsphere sample, and the probe was applied to the imaging of the mouse ear, and human oral cavity in vivo as demonstration. Through imaging an oral cavity, we confirmed that oral abnormalities might be detected by monitoring the thickness of epithelium. Furthermore, this instrumentation can be integrated with other OCT techniques such as polarization-sensitive OCT and speckle variance OCT for providing the additional information in oral cavity including tissue birefringence and vasculature.

8935-55, Session 12

Development of a fiber based Raman probe compatible with interventional magnetic resonance imaging

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Raman spectroscopy has proven to be a powerful tool for discriminating between normal and abnormal tissue types. Fiber based Raman probes have demonstrated the potential for implementing this technique for in vivo tissue diagnostics. Combining Raman spectroscopy with Magnetic Resonance Imaging (MRI) opens up new avenues to MR guided minimally invasive optical biopsy using Raman spectroscopy. Although Raman probes are commercially available, they are not compatible with a MRI environment due to the metallic components used to align the micro-optic components such as filters and lenses at the probe head. Additionally they are not compatible with surgical environments mechanically as factors such as sterility and length of the probe are not addressed in those designs. We have developed an MRI compatible fiber Raman probe with a disposable probe head that can be sterilized. The probe head was specially designed to avoid any material that would cause MR imaging artefacts.. The probe head that goes into patient's body had a diameter <1.5 mm so that it is compatible with biopsy needles and catheters. The probe has been tested in MR environment and has been proven to be capable of obtaining Raman signal while the probe Is under real-time MR guidance.

8935-56, Session 12

Development of a multi-frequency diffuse photon density wave device for the characterization of tissue damage at multiple depths

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The ability to determine the depth and degree of cutaneous and subcutaneous tissue damage is critical for medical applications such as burns and pressure ulcers. The Diffuse Photon Density Wave (DPDW) methodology at near infrared wavelengths can be used to non-invasively measure the optical absorption and reduced scattering coefficients of tissue at depths of several millimeters. A multi-frequency DPDW system with one light source and one detector was constructed so that light is focused onto the tissue surface using an optical fiber and lens mounted to a digitally-controlled stepper motor that sets the distance between light source and detector. A variable RF generator enables the modulation frequency to be selected between 50 to 400 MHz. The ability to digitally control both source-detector separation distance and modulation frequency allows for virtually unlimited number of data points, enabling precise selection of the volume and depth of tissue that will be characterized. Suspensions of intralipid and india ink with known absorption and reduced scattering coefficients were used as optical phantoms to assess device accuracy. Solid silicon phantoms were formulated for stability testing. Standard deviations for amplitude and phase readings were found to be 0.9% and 0.2 degrees respectively, over a one hour period. The ability of the system to quantify tissue damage in vivo at multiple depths was tested in a porcine burn model.

8935-57, Session 12

Development of multiwavelength excitation light source for autofluorescence and photodynamic diagnosis systems

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The study presents the new design of the excitation light source that can stably generate light with center wavelengths of 340nm, 410nm, 450nm, 530nm, 632.8nm and white light for auto-fluorescence(AF) and photodynamic diagnosis(PDD) of cancer in clinics in a single system. The light source consists of Xenon Lamp with 300W power output, light guide module including motorize filter wheel equipped with optical filters with corresponding to wavelength bands, a servo motor, a motorize iris, a cooling system, power supply and optical transmission part for the output light. The transmission part of the light source was developed to collimate the light with desired wavelength into input of fiber optic.

The filter is changed using membrane switch which located in front panel. Each filter is mounted in a black anodized aluminum ring with an outer diameter of Ø1" and a maximum edge thickness of 6.3 mm. Iris was developed to control the output power. The controlling part governs the motor used in the mechanical motion of the optical device and movement of a cooling system. A circuit of switcher voltage regulator was composed inside of the controlling board to protect the circuit from a spark of high voltage generated from an electricity source. As microprocessor, 80C196KC was used. A count of 16 bit and PWM were used to control the speed of the cooling fan. The user's interface constitutes the user's 6 inputs and 26 action indications by enlarging input to port 1 and output port 2 by 8255 Programmable Peripheral Interface(PPI).

8935-58, Session 12

Optical thromboelastography (OTEG): a new approach to evaluate coagulation defects in patients

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Impaired blood coagulation is often associated with increased mortality and hospital length of stay following acute trauma, surgery, and chronic illness. Therefore, the early detection of coagulation defects at the point-of-care is critical in improving recovery and clinical outcome. In this study, we investigate a new approach termed optical

thromboelastography (OTEG), to evaluate coagulation status in real-time, and demonstrate its capability to detect impaired coagulation states in patients. In OTEG, a kaolin-activated blood sample is illuminated by laser light and time-varying laser speckle patterns are captured using a high-speed CMOS sensor. During coagulation, the formation of a platelet-fibrin clot restricts the Brownian motion of light scattering particles and alters the rate of temporal speckle intensity fluctuations. To measure changes in clot viscoelasticity, the temporal speckle intensity auto-correlation function, g2(t), is measured during coagulation, the mean square displacement (MSD) of scattering particles is derived, and the clot viscoelastic modulus, G, is extracted via the generalized Stokes-Einstein relation (GSER). By monitoring changes in G during coagulation the parameters, clotting time (R), clot progression rate (?), and maximum clot strength (MA) are derived. In this study, whole blood samples from 30 patients were analyzed and OTEG coagulation parameters were compared with standard mechanical thromoboelastography (TEG). Our results showed a statistically significant correlation between OTEG and TEG measurements for R-time($R=0.81$, $p<0.001$), ? ($R=0.53$, $p<0.001$), and MA ($R=0.65$, $p<0.001$). These results demonstrate the capability of OTEG for evaluating blood coagulation status and open new opportunities for detecting coagulation defects at the point-of-care.

8935-59, Session 12

Continuous noninvasive in vivo monitoring of intravascular plasma volume and hematocrit changes during hemodialysis in humans: direct comparison with the CRIT-LINE

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We report a new device and algorithm that allows simultaneous monitoring of the hematocrit and plasma volume fraction of blood within the intravascular space of an optically probed volume of skin. Skin is probed with a near infrared laser and simultaneously collecting the Rayleigh and Mie scattered light as one raw signal and the undifferentiated Raman and fluorescence emission as the second raw signal. These signals are combined using six parameters that can be obtained by either direct calculation or empirical calibration to permit monitoring of the blood in human skin (e.g. fingertips). We tested a device based on the algorithm that might be useful in allowing the early detection of blood loss for people who have no external injury but may be hemorrhaging internally. IRB allowed experiments monitoring blood in human fingertip skin in vivo during routine hemodialysis demonstrated good agreement between the experimental device and the CRIT-LINE®, an FDA approved device that is built into the dialysis machine and applies the Twersky algorithm to blood in the dialysis machine (i.e. in vitro). Based on observation of 9 different test subjects, as dialysis removes fluid from the intravascular space causing an increase in hematocrit and a decrease in plasma volume, the CRIT-LINE response is closely emulated (typical per session linear correlation $r2=0.78$, $N=87$, $p<0.0001$) with the new device. Calibration across subjects, the measurement of absolute hematocrit, and potential confounding factors will also be discussed.

8935-60, Session 12

A high sensitivity, anastigmatic, side-facing OCT-guided biopsy needle

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A significant problem in needle biopsy is positioning the needle so as to acquire diagnostically-useful tissue samples. One solution is to incorporate a fiber-optic probe to guide needle placement. Previous designs have typically either used a forward-facing probe which acquires A-scans, but lacks a scanning mechanism to form images; or a side-facing probe which must be removed prior to tissue sampling. We present a novel 12cm long, 14-gauge biopsy needle with a high sensitivity, anastigmatic fibre-optic probe integrated into the tissue channel, which allows simultaneous imaging and tissue sampling.

The distal focusing optics comprise of well-controlled lengths of no-core and gradient-index fiber spliced to the end of a section of single-mode fiber. This is terminated with angle-polished no-core fibre to redirect the light beam, which is encased within a glass capillary to maintain a glass-air interface and achieve total internal reflection. The inherent astigmatism in the fiber probe was overcome using the “focal shift” phenomenon for focused Gaussian beams, achieving equal working distances in both the x and y directions. Fusing the capillary directly to the imaging window minimized parasitic back-reflections, achieving a sensitivity of 110dB. This fiber-optic probe was integrated into the tissue channel of a biopsy needle, allowing the imaging of tissue adjacent to the needle as it is inserted into tissue and prior to sampling. We demonstrate results acquired in a range of tissue samples, distinguishing multiple tissue types.

8935-61, Session 12

Development of a baby friendly non-contact method for measuring vital signs, first results of clinical measurements in an open incubator on a Neonatal Intensive Care Unit

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For infants and neonates in an incubator vital signs, such as heart rate, breathing, skin temperature and blood oxygen saturation are measured by sensors and electrodes sticking to the skin.

This can damage the vulnerable skin of neonates and cause infections. In addition, the wires interfere with the care and hinder the parents in holding and touching the baby. These problems initiated the search for baby friendly ‘non-contact’ measurement of vital signs. Using a sensitive color video camera and specially developed software, the heart rate was derived from subtle repetitive color changes. Potentially also respiration and oxygen saturation could be obtained. A thermal camera was used to monitor the temperature distribution of the whole body and detect small temperature variations around the nose revealing the respiration rate. After testing in the laboratory, two babies were monitored (with parental consent) in the neonatal intensive care unit (NICU) simultaneously with the regular equipment. From the color video recordings accurate heart rates could be derived and the thermal images provided accurate respiration rates. To correct for the movements of the baby, tracking software could be applied. At present, the imaging processing was performed off-line. Using narrow band light sources also non-contact blood oxygen saturation could be measured.Baby friendly non-contact

monitoring of vital signs has proven to be feasible and can be developed into a real time system.

8935-62, Session 12

Minimal resection approaches for lung cancer surgery using intraoperative merged fluorescence imaging system

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Intraoperative Near infrared (NIR) fluorescence imaging system has been developed for operating minimal resection lung cancer surgery. The system of both thoracotomy and thoracoscopic versions provides merged color and NIR fluorescence images simultaneously. The system overcomes poor sensitivity to NIR fluorescence signals aided by custom software in real-time. We conducted preclinical segmentectomy using indocyanine green (ICG). It was found that intersegmental plane was identified clearly through the contrast of the fluorescence signal, therefore, the surgeon could resect the target segment without any injuries to the adjacent vessels and bronchi. Fluorescence signal intensity to background ratio (SBR) for different ICG dosages were estimated and the least dosage was found. Our results indicate that far lower dosage (0.6 mg/kg) compared to that of clinical safety guideline (5.0 mg/kg) enabled successful image-guided lung segmentactomy. We are preparing for clinical translation for human segemtectomy study in the near future.

Conference 8936:
Design and Quality for Biomedical Technologies VI

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8936 Design and Quality for Biomedical Technologies VII

8936-1, Session 1

Simulation of light transport in arthritic- and non-arthritic human fingers

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Rheumatoid arthritis is a disease that frequently leads to joint destruction. It has high incidence rates worldwide, and the disease significantly reduces patient's quality of life due to pain, swelling and stiffness of the affected joints. Early diagnosis is necessary to improve course of the disease, therefore sensitive and accurate diagnostic tools are required.

Optical imaging techniques have capability for early diagnosis and monitoring of arthritis. As compared to conventional diagnostic techniques optical technique is a noninvasive, noncontact and fast way of collecting diagnostic information. However, a realistic model of light transport in human joints is needed for understanding and developing of such optical diagnostic tools. The aim of this study is to develop a 3D numerical model of light transport in a human finger. The model will guide development of a hyperspectral imaging (HSI) diagnostic modality for arthritis in human fingers.

The implemented human finger geometry is based on anatomical data. Optical data of finger tissues is adjusted to represent either an arthritic or an unaffected finger. The geometry and optical data serve as input into a 3D Monte Carlo method, which calculate diffuse reflectance, transmittance and absorbed energy distributions. The parameters of the model are optimized based on HIS-measurements of human fingers.

The presented model serves as an important tool for understanding and development of HSI as an arthritis diagnostic modality. Yet, it can be applied to other optical techniques and finger diseases.

8936-2, Session 1

Optical-thermal response of tissue during photoacoustic imaging

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Photoacoustic (PA) imaging has grown rapidly as a biomedical imaging technique in recent years, with key applications in cancer diagnosis and oximetry. In spite of these advances, the literature provides little insight into optical-thermal light-tissue interactions involved in PA imaging. To elucidate these basic phenomena, we have developed, validated and implemented a three-dimensional numerical model of tissue photothermal response to repetitive laser pulses. The model calculates energy deposition and fluence distributions as well as transient temperature and damage profiles in breast tissue with discrete blood vessels and generalized perfusion. A parametric evaluation of the effect of vessel diameter and depth, as well as wavelength, beam diameter and irradiance, was performed. For a constant radiant exposure level, increasing beam diameter led to a significant increase in subsurface heat generation rate. Increasing vessel diameter resulted in two competing effects – reduced mean energy deposition in the vessel due to light attenuation and greater thermal superpositioning due to reduced thermal relaxation. Maximum temperatures occurred either at the surface or in subsurface regions of the dermis, depending on vessel size and depth. Results are discussed in terms of established exposure limits and levels used in prior studies. While additional experimental and numerical study is needed, numerical modeling represents a powerful tool for elucidating the effect of PA imaging devices on biological tissue.

8936-3, Session 1

Spectrum Correction Considering Light Source Fluctuation for Non-invasive Blood Glucose Sensing

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We are developing the blood glucose estimation algorithm for non-invasive blood glucose sensor by using Mid-Infrared spectroscopy. The purpose of this study is to correct the variation of glucose absorbance spectrum (around 1,180[cm-1] to 980[cm-1] regions) caused by light source fluctuation. Light source fluctuation is the noise from light source itself. This noise makes accurate glucose sensing difficult because it increases or decreases glucose absorbance. Therefore, we correct glucose absorbance by using hemoglobin absorbance around 1,540[cm-1] (Amide II band). Since the amount of hemoglobin is stable at period of time in the blood vessel, it can be thought that hemoglobin absorbance reflects only light source fluctuation. By calculating the proportion of glucose absorbance to hemoglobin one, we can measure relative glucose absorbance. However, hemoglobin absorbance is overlapped with water vapor one. Since water vapor absorbance is easily affected by humidity in the measurement environment, the effect of water vapor should be removed to measure the hemoglobin absorbance accurately. In this study, we firstly remove the effect of water vapor by subtracting water vapor absorbance from sample spectrum. Then, glucose absorbance spectrum is corrected by using hemoglobin one.

In the simulations, blood samples collected from rat are used to verify the effectiveness of the proposed method. Blood samples are measured by using Fourier transform infrared spectroscopy (FT-IR) in the mid-infrared region from 4000[cm-1] to 400 [cm-1] with 4[cm-1] resolutions. From the simulation results, glucose estimation error is successfully reduced by correcting light source fluctuation.

8936-4, Session 1

Oxygenation changes in the skeletal muscles of stroke patients by surface near-infrared spectroscopy

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Introduction: The oxygenation level of a tissue is an important marker of the health of the tissue. Sustained extreme oxygenation levels, in either direction, can cause irreparable damage. In stroke survivors, it has been shown that the blood flow to paretic limbs is significantly reduced compared to a non-paretic limb. We hypothesize that hemodynamic activity in stroke affected muscles is suppressed as compared to normal muscles. In order to measure the hemodynamic activity, we developed an oximetry probe to measure the relative changes in the concentration of oxygenated and reduced hemoglobin (Hb and HbO2) in the tissue using Beer's Law.

Objective: The objective of this study is to examine the difference in muscle blood volume and oxygenation changes in the paretic and non-paretic limbs of stroke patients undergoing rehabilitation with the eventual goal of using the change in oxygenation in a muscle as a diagnostic marker for assessing the progress of rehabilitation. A clear understanding of the way stroke-affected limbs respond to rehabilitation

exercises and a convenient method for monitoring this progress will allow clinicians and therapists to tailor treatments based on individual response.

Methods: We are measuring the change in blood volume and oxygenation in both forearm and calf muscles in stroke survivors' paretic and non-paretic sides. The stroke patients' data is compared to a control group of healthy individuals drawn from the general population. The oximetry probe, consisting of a three-wavelength LED source and four evenly spaced photodetectors, is affixed to the subjects' limbs using an elastic band. One pair of detectors is always oriented along the axis of the muscle, and is taken to provide the more accurate measurement. For the forearm measurements, the forearm is secured so that only the muscles in the region of interest are free to move; each subject undertakes four experiments, two in each forearm, consisting of three exercise levels calibrated to the subject's maximum effort. When measuring the leg muscles, we monitor a group of subjects undergoing walking rehabilitation through the duration of their therapy. In post-processing of the data, we use the output intensity of light at each wavelength to calculate the changes in the relative concentrations of Hb and HbO2 in the muscle.

Results: The results from the forearm control group show a significant ($p<0.05$) change in the blood volume and oxygenation in the muscles of healthy subjects between exercise and rest. During exercise, the muscle oxygenation decreases while the blood volume increases from the baseline value. There is also a significant difference in muscle blood volume and oxygenation levels between a 20% and 40% exercise level. After each period of exercise, we observe that the subjects establish a new baseline (resting) muscle oxygenation and blood volume level. We are currently in the process of studying the stroke survivor population.

8936-5, Session 1

Electrochemical impedance spectroscopy based-on interferon-gamma detection

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Tuberculosis (TB) is an ancient disease constituted a long-term menace to public health. According to World Health Organization (WHO), mycobacterium tuberculosis (MTB) infected nearly a third of people of the world. There is about one new TB occurrence every second. Interferon-gamma (IFN-?) is associated with susceptibility to TB, and interferon-gamma release assays (IGRA) is considered to be the best alternative of tuberculin skin test (TST) for diagnosis of latent tuberculosis infection (LTBI). Although significant progress has been made with regard to the design of enzyme immunoassays for IFN-?, adopting this assay is still labor-intensive and time-consuming. To alleviate these drawbacks, we used IFN-? antibody to facilitate the detection of IFN-?.

An experimental verification on the performance of IGRA was done in this research. We developed two biosensor configurations, both of which possess high sensitivity, specificity, and rapid IFN-? diagnoses. The first is the electrochemical method. The second is a circular polarization interferometry configuration, which incorporates two light beams with p-polarization and s-polarization states individually along a common path, a four photo-detector quadrature configuration to arrive at a phase modulated ellipsometer. With these two methods, interaction between IFN-? antibody and IFN-? were explored and presented in detail in this paper.

8936-6, Session 2

Non-invasive optical volumetric imaging of tissue microstructures and microcirculations in vivo *(Invited Paper)*

Ruikang Wang, Univ. of Washington (United States)

No Abstract Available

8936-7, Session 2

Telemedicine + OCT: toward design of optimized algorithms for high-quality compressed images

Mahta Mousavi, Univ. of California, San Diego (United States); Kristen L. Lurie, Audrey K. Ellerbee, Stanford Univ. (United States); Tara Javidi, Univ. of California, San Diego (United States)

Telemedicine is an emerging technology that aims to provide clinical healthcare at a distance. Among its goals, the transfer of diagnostic images over telecommunication channels has been quite appealing to the medical community. When viewed as an adjunct to biomedical device hardware, one highly important consideration aside from the transfer rate and speed is the accuracy of the reconstructed image at the receiver end. Although optical coherence tomography (OCT) is an established imaging technique that is ripe for telemedicine, the effects of OCT data compression, which may be necessary on certain telemedicine platforms, have not received much attention in the literature. We investigate the performance and efficiency of several lossless and lossy compression techniques for OCT data with the goal to characterize their effectiveness with respect to achievable compression ratio, compression rate and preservation of image quality. Using a combination of in vivo data (e.g., skin, eye) and simulations, we show the relevance of various image-based features (e.g., center of mass, SNR, overall signal level, noise characteristics) to compression performance. Ultimately, we present metrics to assess accuracy in reconstructed images, considerations for the design of optimal compression techniques suited to different clinical applications, and implications of these results on the design choices for the device hardware and operating conditions of OCT systems intended for use in telemedicine.

8936-8, Session 2

Objective assessment of multimodality optical coherence tomography and second-harmonic generation image quality of ex vivo mouse ovaries using human observers

Weston A. Welge, The Univ. of Arizona (United States); Andrew T. DeMarco, The Univ of Arizona (United States); Matthew A. Kupinski, Jennifer M. Watson, Photini S. Rice, Jennifer K. Barton, The Univ. of Arizona (United States)

We have previously obtained co-registered images of 67 mouse ovaries ex vivo using swept-source optical coherence tomography (OCT) and multiphoton microscopy (MPM). The ovaries were treated with 4-vinylcyclohexene diepoxide (VCD) to induce ovarian failure and 7, 12-dimethylbenz(a)anthracene (DMBA) to induce ovarian cancer. The OCT data provide structural detail including epithelial proliferation and follicle structure. The second-harmonic generation (SHG) MPM images visualize collagen structure. The goal of this study is to assess the quality of OCT and SHG images for the binary classification task of normal versus abnormal using human observers.

Expert readers of OCT and SHG images initially trained all observers. The observers were tasked to analyze one SHG or OCT volume at a time, classify the image as normal or abnormal, and to provide a numerical score corresponding to their confidence in their classification. After a break of at least one week, the observers viewed the co-registered SHG and OCT volumes simultaneously and once again classified the images.

From the results of the human observers, we generated several figures of merit, including the receiver operating characteristic (ROC) curve, area under the ROC curve (AUC), and sensitivity and specificity of OCT, SHG, and combined OCT-SHG. With up to 0.93 AUC, the results indicate that both modalities have potential to guide ovarian biopsy.

8936-9, Session 2

Simultaneous estimation of lipid and aqueous thicknesses of the tear film with optical coherence tomography and statistical decision theory

Jinxin Huang, Univ. of Rochester (United States); Eric W. Clarkson, Matthew A. Kupinski, The Univ. of Arizona (United States); Patrice Tankam, Jannick P. Rolland, Univ. of Rochester (United States)

The prevalence of Dry Eye Disease (DED) in the USA is approximately 40 million in aging adults with about $3.8 billion economic burden. However, a comprehensive understanding of tear film dynamics, which is the prerequisite to advance the management of DED, is yet to be realized. To extend our understanding of tear film dynamics, we investigate the simultaneous estimation of the lipid and aqueous layers thicknesses with the combination of optical coherence tomography (OCT) and statistical decision theory.

In specific, we develop a mathematical model for Fourier-domain OCT where we take into account the different statistical processes associated with the imaging chain. A tear film model, which includes a lipid and aqueous layer on top of a rough corneal surface, is the object being imaged. Then we formulate the first-order and second-order statistical quantities of the output of the OCT system, which can generate some simulated OCT spectra. We further implement a Maximum-likelihood (ML) estimator to interpret the simulated OCT data to estimate the thicknesses of both layers of the tear film. Results show that an axial resolution of 1 micron allows estimates down to nanometers scale. We use the root mean square error of the estimates as a metric to evaluate the system parameters, such as the tradeoff between the imaging speed and the precision of estimations. This framework further provides the theoretical basics to optimize the imaging setup for a specific thickness estimation task.

8936-10, Session 3

Development and quality assessment of intra-operative optical imaging systems *(Invited Paper)*

Yu Chen, Univ. of Maryland, College Park (United States)

No Abstract Available

8936-11, Session 3

Novel measure for the calibration of laser Doppler flowmetry devices

Andrey V. Dunaev, Univ. of Dundee (United Kingdom) and State Univ. Educational Scientific Production Complex (Russian Federation); Evgeny A. Zherebtsov, State Univ. Educational Scientific Production Complex (Russian Federation); Dmitrii A. Rogatkin, Moscow Regional Research and Clinical Institute (Russian Federation); Neil Z. Stewart, Sergei G. Sokolovski, Edik U. Rafailov, Univ. of Dundee (United Kingdom)

Metrological support for optical non-invasive diagnostic devices is an urgent task at present. A major issue for laser Doppler flowmetry (LDF) is the need to compare metrological characteristics of individual devices from a manufacturer (and compare various manufacturers) to detect defects and achieve solutions for clinical diagnostics. The most commonly used measures for instrument calibration are phantoms comprising colloids of light-scattering particles which simulate the motion of red blood cells based on Brownian motion. However, such systems do not have sufficient accuracy or stability, and also cannot check the operational registration of rhythmic components of perfusion (0.0095-1.6 Hz).

To solve this problem, we propose the design of a novel measure, built on the principle of simulation of moving particles using an electromechanical transducer, in which a precision piezoelectric actuator can be used (e.g., P-602.8SL with maximum movement under 1 mm). In this system, Doppler shift is generated in a layered structure of different solid materials with different optical light diffusing properties, comprising of a fixed upper layer (e.g., thin polymer film company «Rosco») plus an oscillating fluoroplastic disk. Preliminary studies on this experimental setup using the LDF-channel of a LAKK-M system demonstrated the detection of the linear portion (0-10 Hz with maximum signal corresponding to Doppler shift of about 5 kHz) of LDF-signal from the oscillating frequency of the moving layer. The results suggest the possibility of applying the considered structure as a measure for calibration of LDF devices.

8936-12, Session 3

Quantification of air flows produced by medical equipment disturbing the clean air in the field of surgery using large field background subtraction imaging

Rudolf M. Verdaasdonk, Niek van Asperena, Albert J. van der Veen, Keith S. Cover, John M. Klaessens, Peter W. P. Vandertop, Vrije Univ. Medical Ctr. (Netherlands)

Medical equipment is usually cooled by an internal air fans exiting the chassis on the side or the rear. These air exhausts can induce a major disturbance of the laminar flow of clean air in the operating room (OR) specially created above the field of surgery to prevent contaminations. A special large field air flow visualization technique has been developed to study and quantify the air flow around equipment used in the OR that might potentially disturb the laminar flow in the operating field. By digital subtraction of the fine line pattern in the background of the field of interest, optical distortions induced by small density gradients in flowing air can be visualized with high contrast and sensitivity. Short bursts of air contrast were induced at the exhaust frame of medical equipment and followed in time. Specially developed analysis software calculated speed and direction of the air flow.

Measurements revealed that the exhaust of cooling air of critical equipment like an operating microscope and surgical navigation system were blowing air at high speed into the clean laminar field. Also devices warming the patient with hot air can blow into the ‘clean’ field of surgery.

Large field background Schlieren digital subtraction imaging shows to be a sensitive and quantitative technique to study air flows. The awareness of the disturbance of the clean laminar air flow should lead to guidelines to improve the design and positioning of medical equipment in the OR to reduce infections.

8936-13, Session 3

Fresh versus frozen tissue preparations for quantitative fluorescence lifetime imaging of NADH and FAD

Alex J. Walsh, Rebecca S. Cook, Melissa C. Skala, Vanderbilt Univ. (United States)

Optical endpoints show promise as biomarkers of cancer malignancy and anti-cancer drug response. However, studies are often limited to measurements from in vivo or freshly excised tissue, which presents

limitations in amassing the large number of samples to thoroughly validate optical techniques. Banks of frozen tissues linked with comprehensive patient history could be used to rapidly validate optical techniques, if the endpoints are robust in frozen tissues. We performed multi-photon fluorescence lifetime imaging of NADH and FAD on frozen-thawed tissues and matched fresh tissues to investigate the use of frozen tissues in fluorescence lifetime studies. Furthermore, we compared anti-cancer drug induced changes in NADH and FAD fluorescence lifetime values from organoids generated from fresh and frozen-thawed tissues. Xenograft tumors and fresh clinical tumor samples were obtained and divided into three pieces: one was imaged immediately; a second was dissociated into organoid cultures, treated with anti-cancer drugs, and imaged at 24, 48, and 72 hours; the third was flash-frozen in liquid nitrogen, stored in a -80 freezer, thawed, imaged, and processed into organoids. Organoids generated from frozen-thawed tissues maintained viability. Optical endpoints of drug treatment response of organoids from frozen-thawed tissue agreed with changes observed in organoids generated from matched fresh tissues ($p<0.05$). These results suggest that frozen-thawed tissue may be an alternative for fresh tissues in fluorescence lifetime studies of intact tissues and organoid cultures, as long as proper controls are included to measure a relative change in response to a perturbation.

8936-14, Session 3

Reproducibility analysis of measurements with a mechanical semiautomatic eye model for evaluation of intraocular lenses

Elisabet Rank, Lukas Traxler, Fachhochschule Technikum Wien (Austria); Kirsten Lux, Christian Krutzler, Integrated Microsystems Austria GmbH (Austria); Andreas Drauschke, Fachhochschule Technikum Wien (Austria)

Mechanical eye models are used to validate ex vivo the optical quality of intraocular lenses (IOLs). The quality measurement and test instructions for IOLs are defined in the ISO 11979-2 norm. However, it was mentioned in literature that these test instructions could measure inaccurate in case of some modern IOL designs.

Reproducibility of alignment and measurement processes are presented, performed with a semiautomatic mechanical ex vivo eye model based on optical properties published by Liou & Brennan in the scale 1:1. The cornea, the iris aperture and the IOL itself are separately changeable within the eye model. The adjustment of the IOL can be manipulated by automatic decentration and tilt of the IOL in reference to the optical axis of the whole system, which is defined by the connection line of the central point of the artificial cornea and the iris aperture.

With the presented measurement setup two quality criteria are measurable: The modulated transfer function MTF and the Strehl ratio S. First the reproducibility of the alignment process for definition of initial conditions of the lateral position and tilt in reference to the optical axis of the system is investigated.

Furthermore different IOL holders are tested related to the stable holding of the IOL. The measurement is performed by a before-after comparison of the lens position using a typical decentering and tilt tolerance analysis path. MTF and Strehl ratio before and after this tolerance analysis are compared and requirements for lens holder construction are deduced from the presented results.

8936-15, Session 3

A novel yet effective motion artefact reduction method for continuous physiological monitoring

Abdullah Alzahrani, Sijung Hu, Vicente Azorin-Peris, Roy Kalawsky, Loughborough Univ. (United Kingdom); Xiaolong Zhang, Changqing Liu, Loughborough Univ (United Kingdom)

This study presents a non-invasive and wearable optical technique to continuously monitor vital human signs as daily demanded by ageing population increasing and personal healthcare. The study aims to research into an effective way to capture human critical physiological parameters, i.e., oxygen saturation (SaO2%), heart rate, respiration rate, body temperature, heart rate variability through a well contracted and wearable patch sensor together with a real-time, reduce noise and secure wireless communication functionalities. The work presents the first step of this research; an automatic noise cancellation method using a 3-axes MEMS accelerometer to recover signals corrupted by body movement as one of the biggest sources of motion artefacts. These kinds of motion artefacts could be reduced by an appropriate electronic design and development for self-cancellation noise and stability of the sensor. The signals from the acceleration and the Opto-electronic sensor are highly correlated thus the desired pulse waveform with rich bioinformatics signals retrieved with reduced motion artefacts. The preliminary results from the bench tests and the laboratory set-up demonstrate that the goal of the high performance wearable opto-electronics is viable and feasible.

8936-16, Session 4

Per-cutaneous single-fiber reflectance spectroscopy for post-morterm evaluation of mineral degeneration in canine intervertebral disc and in vivo assessment of liver steatosis in a rat model

(Invited Paper)

Daqing Piao, Nigar Sultana, Kelci McKeirnan, Melanie A Breshears, G. Reed Holyoak, Jerry W Ritchey, Oklahoma State University (United States); Anqi Zhang, Johns Hopkins University (United States); Kenneth E Bartels, Oklahoma State University (United States)

Reflectance spectroscopy using light in the visible and near-infrared (VIS/NIR) spectrum has been used broadly for non-invasive or minimally invasive sensing of tissue optical properties. VIS/NIR reflectance spectroscopy via a needle-probe or an applicator of very small cross-sectional profile has also been employed for surface, interstitial or intra-endoscopic measurements of a variety of pathological conditions. This study performs single-fiber reflectance spectroscopy (SfRS) percutaneously for the potential to evaluate mineral degeneration in intervertebral discs in dogs and to assess the changes of optical characteristics of rat liver associated with the modeling of fatty liver. Percutaneous SfRS was performed on a total of 28 intervertebral discs of three dogs post-mortem, and the discs were imaged prior to percutaneous SfRS by radiography and CT, and harvested after percutaneous SfRS for histopathologic examinations. The overall numbers of degenerated discs and normal discs were 20 and 8, respectively. Of the total 28 discs the CT had an overall positive predictive value (PPV) of 78.8% and negative predictive value (NPV) of 44.4%. Of the total 28 discs the radiography has an overall PPV of 100% and NPV of 30.4%. The receiver-operating-characteristic analysis of the SfRS measurement was performed on 24 discs that had a mineralization not greater than 50% whereby a correlation between SfRS intensity indexed from the same model of sensing and the level of mineralization

could be inferred. Percutaneous SfRS under ultrasound guidance was also performed on 6 rats, with 2 controls and 4 tests, over a period of 99 days of fatty-liver modeling.

8936-17, Session 4

Hyperspectral spatial frequency domain optical tomography technique for quantitative three-dimensional reconstruction of tissue properties

Robert H. Wilson, Elliott Kwan, Haotian Cui, Beckman Laser Institute and Medical Clinic (United States); Tomasz S. Tkaczyk, Rice Univ. (United States); Bernard Choi, Anthony J. Durkin, Bruce J. Tromberg, Beckman Laser Institute and Medical Clinic (United States)

Hyperspectral optical tomography, using images acquired at many wavelengths, can create quantitative three-dimensional maps of optical properties of biological tissues, since the different wavelengths sample different depths in the tissue. Spatial frequency domain (SFD) optical imaging, in which light of different spatial frequencies is projected onto tissue, can also be employed to reconstruct three-dimensional tissue property maps, since the different spatial frequencies are known to sample different depths in the tissue. Here, we develop a quantitative optical tomography technique including both hyperspectral and SFD methods for accurate reconstruction of tissue properties. This method can be employed to reconstruct absorption and scattering properties of tissue and use the reconstructed properties to correct tissue fluorescence for attenuation, enabling quantitative 3D maps of fluorophore concentrations. Toward this aim, we validated components of the tomography technique by imaging tissue-simulating phantoms with inclusions that mimicked tissue features such as buried tumors and blood vessels. Images were acquired in both the visible and near-infrared ranges of the spectrum, with wavelengths ranging from 420 nm to 970 nm. Optical tomography algorithms using diffusion theory and the Rytov approximation were employed to reconstruct three-dimensional images of the buried inclusions. Experiments were performed to determine the ability of the technique to accurately quantify the contrast level between the inclusion and the surroundings. Experiments were also performed to determine the limits on the inclusion depth, shape, resolution and contrast level that could be reconstructed in three dimensions.

8936-19, Session 4

Sensitive time-correlated single photon counting enables efficient singlet oxygen detection

Uwe Ortmann, Manoel Veiga, Steffen Ruettinger, Sebastian Tannert, Felix Koberling, Christian Litwinski, Matthias Patting, Marcus Sackrow, Michael Wahl, Rainer Erdmann, PicoQuant GmbH (Germany)

Single photon counting based data acquisition has proven to yield a major sensitivity increase in the optical evaluation of pharmaceuticals and biotechnology products. We will show for the first time that a state of the art time-correlated single photon counting (TCSPC) based fluorescence lifetime spectrometer is able to quantify singlet oxygen generation and to characterize the singlet oxygen phosphorescence decay. This makes TCSPC based fluorescence lifetime spectrometers a valuable tool for studying photosensitizers widely used for example in photodynamic therapy (PDT). The detection of the faint singlet oxygen phosphorescence signal has been made possible by using a special burst mode for the pulsed laser excitation and a new generation of TCSPC electronics with a significantly reduced dead-time which enables efficient multi-stop photon detection.

Thanks to a recently developed integrating sphere add-on we are also able to measure fluorescence quantum yields with the same instrument. Leveraging the possibility to measure fluorescence lifetime in conjunction with quantum yield, we performed a systematic investigation of the relation between reduced fluorescence emission and different contributions of dynamic and static quenching processes.

Furthermore, since quenching normally does not affect the radiative rate constant, this combined set-up allows to verify the accuracy of the extracted lifetime. Especially for very short lifetimes in the range of the instrument response function the presented method allows to assess whether the proper fluorescence lifetime was extracted.

8936-20, Session 4

A digital broad bandwidth frequency-domain Diffuse Optical Spectroscopy (dDOS) system for multiplexed measurements in turbid media

Justin Jung, Raeef Istfan, Darren M. Roblyer, Boston Univ. (United States)

Frequency domain diffuse optical spectroscopy (DOS) is used to perform non-invasive functional imaging for applications including tumor detection and therapy monitoring. Techniques for both signal synthesis as well as amplitude and phase measurements have traditionally relied on complex RF circuit design and/or expensive test equipment. We previously demonstrated the use of a new digital Diffuse Optical Spectroscopy (dDOS) system that employs both digital signal synthesis and detection. 14-bit, 1 giga sample per second (GSPS) direct digital synthesizers (DDS) are used as frequency sources for laser diodes at six near-infrared wavelengths. At each wavelength, the signal is swept between 50MHz and 400MHz and collected signals are directly sampled with 12-bit, 3.6 GSPS analog-to-digital converter (ADC). Proof of concept experiments demonstrated a 3.6% and 2.8% agreement in absorption and scattering coefficients compared with a network-analyzer based system. Here we present the next generation dDOS system, which has been improved for measurement throughput towards clinical use. The system is controlled through a custom field-programmable gate array (FPGA) design that allows for rapid measurements and versatility in sweet parameters and sample lengths. Furthermore, the system is capable of wavelength multiplexing, with six DDS sources simultaneously modulating the six laser diodes at offset RF frequencies with simultaneous signal collection by the ADC. Phase and amplitude measurements at each wavelength are extracted during post processing using Fourier analysis. dDOS is a promising alternative to more traditional frequency domain DOS techniques and may reduce system complexity, lower costs, provide smaller device footprints, and increase measurement versatility.

8936-21, Session 5

Special imaging techniques in tissue models for validation, safe application, education, and improvement of medical devices *(Invited Paper)*

Rudolf M. Verdaasdonk, Albert J. Van der Veen, John M. Klaessens, Vrije Univ. Medical Ctr. (Netherlands)

The testing of medical devices in simulated clinical conditions is essential to optimize the settings and for a safe application. Also the basic understanding of the (physical) mechanism of action induced of the medical device in the patient is of major importance. Over the last 20 years many medical devices have been studied during interacting with tissues phantoms using specially developed high speed, high contrast and thermal imaging techniques. Schlieren techniques were applied for real-time visualization of dynamic temperature gradients with high spatial

and temporal resolution (up to 2000 f/s) to study the interaction of e.g. lasers, electro-surgery and RF ablation devices. Mechanical interactions such as explosively expanding and imploding vapor bubbles, cavitation , pressure waves and shock waves induced by e.g. pulsed lasers, waterjet, cavitation surgical and ultrasonic devices, were studied with high speed imaging and contrast enhancement techniques down to nanosecond resolution. The methods provided qualitative imaging and enable relative comparison of effects changing parameters like energy, pulse duration, repetitive exposure etc. These results have contributed to a better understanding of the mechanism of action, safety and improvement of the application and the development of new medical devices. Although the testing was performed in model tissues, the video clips have proven to be of great value for educating researchers, surgeons, nurses, and students on the use of medical devices in patients.

8936-22, Session 5

Quantitative assessment of biophotonic imaging system performance with phantoms fabricated by rapid prototyping

Jianting Wang, Univ. of Maryland, College Park (United States); James Coburn, U.S. Food & Drug Administration (United States); Nicholas Woolsey, Chia-Pin Liang, Univ. of Maryland, College Park (United States); Du Vinh Nguyen Le, McMaster Univ. (Canada); Jessica Ramella-Roman, The Catholic Univ. of America (United States); Yu Chen, Univ. of Maryland, College Park (United States); Joshua Pfefer, U.S. Food & Drug Administration (United States)

In biophotonic imaging, turbid phantoms that are low-cost, biologically-relevant and durable are desired for standardized performance assessment. Such phantoms often include inclusions of varying depths and sizes in order to quantify key image quality characteristics such as depth penetration, sensitivity and contrast detectability. The emerging technique of rapid prototyping with three-dimensional (3D) printers provides a potentially revolutionary way to fabricate these structures. Towards this goal, we have characterized the optical properties and morphology of phantoms fabricated by two 3D printing approaches: thermosoftening and photopolymerization. Material optical properties were measured by spectrophotometry while the morphology of phantoms incorporating 0.2-1.0 mm diameter channels was studied by micro-CT, optical coherence tomography (OCT) and optical microscopy. A near-infrared absorbing dye and nanorods at several concentrations were injected into channels to evaluate detectability with a near-infrared hyperspectral reflectance imaging (HRI) system (650-1100 nm). Phantoms exhibited biologically-relevant scattering and low absorption across visible and near-infrared wavelengths. Although limitations in resolution were noted, channels with diameters of 0.4 mm or more could be reliably fabricated. The most significant problem noted was the porosity of phantoms generated with the thermosoftening-based printer. The aforementioned three imaging methods provided a valuable mix of insights into phantom morphology and may also be useful for detailed structural inspection of medical devices fabricated by rapid prototyping, such as customized implants. Overall, our findings indicate that 3D printing has significant potential as a method for fabricating well-characterized, standard phantoms for medical imaging modalities such as HRI.

8936-23, Session 6

Intra-operative optical imaging of breast tumor margins *(Invited Paper)*

Nimmi Ramanujam, Duke Univ. (United States)

Approximately 192,370 new invasive breast cancers are diagnosed annually, and the treatment for most of these is a procedure known as breast conserving surgery (BCS), where the tumor and a surrounding layer disease-free tissue, or “margin” is excised. The standard of care at Duke University Medical Center requires at least a 2mm margin of normal tissue, since studies have shown that margins less than 2mm have the potential for local recurrence. The current standard of care stipulates that assessment of the margin status is made post-operatively by a pathologist, eliminating any possibility of additional tissue removal during the first surgery, and requiring a large number of patients to undergo a second re-excision surgery. It has been the goal of my research group to develop rapid, non-invasive systems for intraoperative margin assessment using diffuse reflectance spectroscopy (DRS). Over the last 10 years, we have advanced the technology from collecting data with a crude, full-spectrum multi-placement system, to a large clinical study with a highly multiplexed full-spectrum system, and finally, to a reduced-wavelength, ultra-portable multi-channel system that still achieves high spatial resolution.

In this talk I will discuss three specific embodiments of our margin assessment technology. The first is an 8-channel optical spectral imaging system consisting of a xenon lamp, a fiber-based imaging probe, a spectrograph, and a CCD. An inverse Monte Carlo model was used to extract specimen tissue composition, based on optical properties, from the measured spectral image. The ratio of ??carotene (fat) to scattering (fibroglandular tissue) and the ratio of hemoglobin (blood) to scattering were used diagnostically in an initial 48-patient study, resulting in a sensitivity of 79% and a specificity of 67% in determining margin status. This technology required up to eight placements to survey a complete margin, which prevented intraoperative assessment of the entire specimen.

To improve acquisition speed, a second full spectral device has been constructed with 49 channels covering a 5cm x 5cm surface area (which should be sufficient to cover the largest expected margins), and a native resolution of 6mm. Raster scanning has improved the resolution to 1.2 mm, allowing the visualization of much smaller tissue features, and minimizing the risk of missing focal margin positivity. A large 200-patient study is currently underway with this device.

Finally, we have developed a novel miniature embodiment of the device with improved size, speed, resolution, and control of pressure. A novel imaging detector was developed utilizing an array of 16 (4x4) custom annular photodiodes (PDs) with etched apertures at each PD center for back-illuminated source light [5]. PD spacing was chosen to be 4.5mm as higher density embodiments were adversely affected by crosstalk from source light from neighboring pixel apertures. The light source is a Xenon lamp utilizing 8 narrowband (10nm) spectra with optimized center frequencies, determined using a genetic algorithm shown to minimize the number of needed wavelengths while preserving optical property extraction accuracy. The imaging components are attached to a custom pressure-sensitive imaging platform in which user specified pressures are maintained at the specimen/imaging array interface. A study of the effects of pressure using layered animal tissue revealed that 2mm of fatty tissue is substantially compressed at pressures as low as 16mmHg. A second large-scale patient study is underway with

this device.

8936-24, Session 6

Design and validation of Intra-operative guidance of surgery *(Invited Paper)*

Anita Mahadevan-Jansen, Vanderbilt Univ. (United States)

No Abstract Available

8936-25, Session 6

Intraoperative imaging and fluorescence image guidance in oncologic surgery using a wearable fluorescence goggle system

Suman B. Mondal, Washington Univ. in St Louis (United States); Shengkui Gao, Washington Univ. in St. Louis (United States); Nan Zhu, The Univ. of Arizona (United States); Gail P. Sudlow, Walter J. Akers, Washington Univ. in St Louis (United States); Rongguang Liang, The Univ. of Arizona (United States); Viktor Gruev, Samuel Achilefu, Washington Univ. in St Louis (United States)

We have developed a wearable, fluorescence goggle based system for intraoperative imaging of tumors and image guidance in oncologic surgery. Our system can detect fluorescence from cancer selective near infra-red (NIR) contrast agent, facilitating intraoperative visualization of surgical margins and tumors otherwise not apparent to the surgeon. The fluorescence information is displayed directly to the head mounted display (HMD) of the surgeon in real time, allowing unhindered surgical procedure under image guidance. This system has the potential of improving surgical outcomes in oncologic surgery and reduce the chances of cancer recurrence.

8936-26, Session 6

Optical design of fluorescence imaging system for image guided surgery

Nan Zhu, College of Optical Sciences, The Univ. of Arizona (United States); Shengkui Gao, Suman B. Mondal, Viktor Gruev, Samuel Achilefu, Washington Univ. in St. Louis (United States); Rongguang Liang, College of Optical Sciences, The Univ. of Arizona (United States)

In this presentation, we will discuss optical design of fluorescence imaging system for image guided surgery. We will also present the system evaluation of the prototype system.

8936-27, Session 6

Scanning fiber endoscope with multi fluorescence-reflectance imaging channels for guiding biopsy

Chenying Yang, Vivian W. Hou, Leonard Y. Nelson, Richard S. Johnston, C. David Melville, Eric J. Seibel, Univ. of Washington (United States)

Background: Many early cancerous conditions are treatable but invisible to conventional endoscopy. The challenge for the next generation of endoscope technology is to provide image contrast for pre-cancerous lesions before they become invasive. Furthermore, as many tumors express multiple cell surface markers and these molecular signatures are heterogeneous across patients, simultaneous imaging of numerous different molecular targets is important for increasing the sensitivity of cancer diagnosis. For molecular imaging of pre-cancerous tissue, a wide-field, concurrent multi-spectral fluorescence-reflectance scanning fiber endoscope (SFE) was developed.

Methods: The SFE device was designed and manufactured for concurrent multi-spectral fluorescence molecular endoscopy, with an additional reflectance channel for navigation and Distance Compensation. Test targets were fabricated for the 3 channels of laser based fluorescence imaging (laser excitation at 448,488, 642nm). Using these targets, the multi-spectral SFE was evaluated for detectability of Coumarin, Fluorescein (FITC), and Cyanine (Cy5). Furthermore, a multi-spectral fluorescence-reflectance intraoperative navigation approach was developed for red-flagging and guiding biopsy.

Results: The multi-spectral SFE's performance was evaluated. Concurrent (30Hz), wide-field (80°) and high resolution (50um) imaging was demonstrated on all channels. Using targets matching in vivo dye concentration (1 uM), the multispectral SFE demonstrates high-contrast fluorescence detectability. Meanwhile, intraoperative navigation with ranked red-flagging was achieved with a gray-scale reflectance background and concurrent multi-spectral fluorescence imaging.

Conclusions: Preliminary results show that the newly developed SFE technology and methodologies have met the clinical challenges. This new equipment is ready for upcoming clinical/pre-clinical image applications for detection of early cancer and guiding biopsy using molecular imaging.

8936-18, Session 7

A novel combined frequency-domain near-infrared spectroscopy and diffuse correlation spectroscopy system

Erin M. Buckley, Stefan A. Carp, Pei-Yi Lin, Massachusetts General Hospital (United States); Haruo Nakaji, Canon U.S.A., Inc. (United States); Jay Dubb, Massachusetts General Hospital (United States); Dennis M. Hueber, ISS, Inc. (United States); Patricia Ellen Grant, Mathieu Dehaes, Children's Hospital Boston (United States); David A. Boas, Maria Angela Franceschini, Massachusetts General Hospital (United States)

We present a novel system combining frequency domain near-infrared spectroscopy (FD-NIRS) and diffuse correlation spectroscopy (DCS) in a small, portable instrument and summarize its performance characteristics. FD-NIRS and DCS employ near-infrared light to non-invasively probe static and dynamic optical properties of cortical brain tissue microvasculature. Multi-distance FD-NIRS quantifies absolute cerebral oxygen saturation (SO2), oxygen extraction fraction (OEF), and blood volume (CBV) using photon diffusion theory. The FD-NIRS portion of the instrument consists of 16 RF (110MHz) modulated laser diodes operating at 8 different wavelengths ranging from 670-830 nm and 2 photomultiplier tube detectors (PMT) for heterodyne detection.

Diffuse correlation spectroscopy (DCS) is a relatively new technology that quantifies an index of regional cerebral blood flow (CBFi) by fitting the temporal intensity autocorrelation function of the detected light to the solution of the correlation diffusion equation. The DCS component of the instrument consists of a long-coherence length 852nm laser, 4 low dark-count photon counting avalanche photodiodes, and a custom-made 256-tau correlator.

By combining CBFi obtained from DCS with OEF obtained from FD-NIRS, we can quantify an optical index of cerebral oxygen metabolism (CMRO2i), a parameter that is critical to assessing brain health in critically-ill neonates. A notch filter in front of the PMTs, along with in-house designed software and a custom-made optical probe enables simultaneous FD-NIRS/DCS measurements, which are critical for fast, continuous bedside monitoring of cerebral hemodynamics in neonatal populations.

8936-28, Session 7

Compact spectral-polarization imaging sensor *(Invited Paper)*

Viktor Gruev, Washington Univ. in St. Louis (United States)

Current division-of-focal-plane polarization imaging sensors can perceive intensity and polarization in real time with high spatial resolution, but

are oblivious to spectral information. In this paper, I will describe a novel spectral-polarization imaging sensor that uses the division-of-focal-plane paradigm. We have designed the sensor by the monolithic integration of pixelated nanowire linear polarization filters with a spectrally selective imaging array. The sensor can therefore simultaneously acquire spectral and polarization information in a scene with high spatial and temporal resolution. Furthermore, both spectral and polarization information is co-registered in hardware by virtue of the imaging sensor architecture. The sensor has a pixel pitch of 5 μm and an imaging array of 168 by 256 elements. Each element comprises spectrally sensitive vertically stacked photodetectors integrated with a 140 nm pitch nanowire linear polarizer. The sensor has a maximum measured SNR of 45 dB, extinction ratio of ~3.5, QE of 12%, and linearity error of 1% in the green channel. I will also discuss some of the image processing algorithms developed for this custom imaging architecture used to increase the accuracy of captured spectral and polarization information. I will conclude with examples where this technology has been utilized.

8936-29, Session 7

Challenges and promises in quantitative label-free hyperspectral confocal imaging

Daniel J. Stark, Ji Youn Lee, Robert Chang, National Institute of Standards and Technology (United States); Fuyuki Tokumasu, National Institutes of Health (United States); Kimberly A. Briggman, National Institute of Standards and Technology (United States); Do-Hyun Kim, U.S. Food & Drug Administration (United States); Jeeseong Hwang, National Institute of Standards and Technology (United States)

In label-free absorption-based wide-field microscopy, the local concentration of light-absorbing molecules may directly be calculated using the Lambert-Beer law with knowledge of the local thickness of the sample. However, in confocal microscopy, the high numerical aperture of focusing and collection optics and the index-mismatching interfaces of different materials along light paths add “artifact” contrast to the image. Here, we introduce a phantom, a wedge-shape cavity filled with a hemoglobin solution with known concentration, to develop a model-based image interpretation technique in an effort to identify and quantify the “artifacts” and then obtain the wavelength-dependent absorption coefficient of the hemoglobin solution. This study advances the knowledge of applying the hyperspectral confocal imaging technique towards label free, quantitative chemical mapping of biomarkers in cells and tissues with complex geometries.

8936-30, Session 7

Design of an extended field of view two-photon light-sheet microscope for live embryo imaging

Ming Zhao, Leilei L. Peng, College of Optical Sciences, The Univ. of Arizona (United States)

We built a two-photon light-sheet microscope that performs cellular-resolution video-rate imaging in deep tissue over an extended field of view of 500-by-500 microns. The extended field of view is obtained by using a Bessel beam scanning laser sheet, generated by a special light modulator and a galvo-mirror. A special live embryo mounting method is developed for minimalizing vibration and aberration at 0.5-micron lateral resolution while maintains undisturbed embryo development. The system is also design with zooming magnification, which allows easy switching between full field-of-view for embryo positioning and high-resolution view of region of interest. The system is applied to deep tissue imaging of live zebrafish embryos and drosophila embryos. Deep penetrating 3D imaging of GFP labeled structures and video-rate 2D imaging of heartbeat and blood circulation were demonstrated.

8936-31, Session 7

System design and evaluation of the array confocal fluorescence microscope

Shaun Pacheco, Rongguang Liang, College of Optical Sciences, The Univ. of Arizona (United States)

The scanning speed for conventional confocal fluorescence imaging system is limited due to several factors. To improve the speed of scanning, we develop an array confocal fluorescence microscope (ACFM) that can image large 3D volumes faster than conventional confocal microscopes over a large Field of view (FOV). This presentation will discuss the design and evaluation of the array confocal fluorescence microscope

8936-32, Session 7

Giga-pixel on-chip microscopy and tomography using lensfree holography with color image sensors

Wei Luo, Alon Greenbaum, Serhan O. Isikman, Ahmet F. Coskun, Aydogan Ozcan, Univ. of California, Los Angeles (United States)

In addition to their compactness and field-portability, lensfree on-chip holographic imaging modalities possess much larger fields-of-view (FOV) compared to conventional optical microscopes. By raising the objects-of-interest slightly above the imager chip (e.g., ~0.1-2mm), lensfree on-chip holography provides the ease of implementing source-shifting based pixel-super-resolution which reduces the effective pixel-size, improving the resolution of the reconstructed images while also maintaining the large FOV of the on-chip microscope.

To push the limits of this high-throughput imaging technique, here we report a giga-pixel lensfree holographic microscopy and tomography platform which utilizes a low-cost color CMOS imager for hologram acquisition. By developing an optimized pixel-super-resolution method, which takes into account the physical 2D responsivity distribution within each color pixel, our lensfree microscope achieves a lateral resolution of ~225nm using 372nm illumination, which is equivalent to a numerical aperture of ~0.8 across a large FOV of ~21mm2, achieving an effective pixel count of ~1.6Billion. Using this holographic imaging platform, we scanned the illumination angle across two orthogonal axes to perform optical tomography with a resolution of ~0.35μm?0.35μm?~2μm (x, y and z, respectively) at 530nm illumination. With an effective voxel volume of ~0.03μm3 across an imaging volume of ~5mm3, this lensfree tomographic microscope can provide a total voxel count of >150Billion. To demonstrate its lateral and three-dimensional resolving capabilities, multi-walled carbon nanotubes, blood smears and wild-type C. elegans nematode have been imaged. This work paves the way for developing ultra-high throughput, compact, and cost-effective imaging modalities for biomedical applications in e.g., point-of-care and field settings.

8936-33, Session 8

Optical systems engineering and manufacturing for biomedical technologies: examples and applications

Peter Triebel, Hilmar Straube, Lutz Reichmann, Marco Bornschein, Helmut Bernitzki, Kay-Uwe Klepzig, JENOPTIK Optical Systems GmbH (Germany); Ingolf Reischel, JENOPTIK Polymer Systems GmbH (Germany)

Biomedical imaging and sensing applications are widely used in different fields of applications like Endoscopy, Ophtalmology, DNA Sequencing, UHTS/Drug Discovery and CAD/CAM prosthesis generation. The impact

of the following approach can be achieved by the combination of classical optical elements like lenses and filters together with diffractive and PMMA-based optics. Classical optical aspheres and cylindrical elements are used for miniaturizing and combination of functionality. Diffractive elements produced with electron-beam or gray-scale technologies are widely used for manipulation of phase and or intensity profiles in the way of patterning or beam shaping. PMMA based optics can be used for lab-on-a chip components.

The systems design of complex opto-electronical or opto-mechanical systems based on the optical design with Ray-tracing or wave-optical approaches together with FEM analysis. Thus simulation method which allows simulation of the thermal behavior of optical systems starting with a description of the temperature gradient affecting the optics, generating transient temperature profile and surface deformation using FEM method, uploading the FEM results to optics design software, generating three-dimensional refraction index distribution and calculate the propagation with respect to discrete time steps.

Due to the integration and assembly of optical mechanical manipulators provide a long term stability and accuracy to align microoptical elements like micro lens arrays and diffractive beam shapers as well opto-electronical elements. The example of a complex system layout with respect to single components and their specifications will be presented in his paper.

8936-34, Session 8

Optical testing of progressive ophthalmic glasses based on galvo mirrors

Stephan Stuerwald, Fraunhofer-Institut für Produktionstechnologie (Germany); Robert Schmitt, RWTH-Aachen University (Germany)

In production of ophthalmic freeform optics like progressive eyeglasses, the specimens are tested according to a standardised method which is based on the measurement of the vertex power on usually less than 10 points. For a better quality management and thus to ensure more reliable and valid tests, a more comprehensive measurement approach is required. For Shack Hartmann Sensors (SHS) the dynamic range is defined by the number of micro-lenses and the resolution of the imaging sensor. Here, we present an approach for measuring wavefronts with increased dynamic range and lateral resolution by the use of a scanning procedure. Therefore, the proposed innovative setup is based on galvo mirrors that is capable of measuring the vertex power with a lateral resolution below one millimetre since this is sufficient for a functional test of progressive eyeglasses.

Expressed in a more abstract way, the concept is based on a selection and thereby encoding of single sub-apertures of the wave front under test. This allows to measure the wave fronts slope consecutively in a scanning procedure. The use of a high precision galvo systems allows high lateral resolution as well as a significant fast scanning ability. The measurement concept and performance of this method will be demonstrated for different spherical and freeformed specimens like progressive eye glasses. Furthermore, approaches for calibration of the measurement system will be characterised comprehensively and the optical design of the detector will be discussed in detail.

8936-35, Session 8

Comparison of digital holographic lenses to increase the depth of focus

Alexis Vazquez-Villa, Instituto Nacional de Astrofísica, Óptica y Electrónica (Mexico); Ruikang K. Wang, Univ. of Washington (United States); Jorge Castro-Ramos, José Alberto Delgado-Atencio, Instituto Nacional de Astrofísica, Óptica y Electrónica (Mexico)

Introduction. Optical systems like optical coherence tomography, confocal microscopy, among others, exist a trade-off between lateral resolution and depth of focus (DOF) by the relation DOF=?/2NA?, where ? is the wavelength and NA is the numerical aperture. Several different approaches have been studying by different groups in order to improve the depth of focus, for example, digital processing, refractive optical elements like axicons, a combination of the above such as wavefront coding, or diffractive optical elements such as fractal zone plates. In this study we compare three digital holographic diffractive lenses in terms of the depth of focus and the intensity, when is compare with a Fresnel lens, using a broadband source.

Materials and methods. We generated computer holograms using the phase of a Fresnel lens, axicon, axilens and multiplexed lens. The case for focal distances of f1=180mm and f2=50mm were analyzed. The holograms were displayed in a spatial light modulator and illuminated using a supercontinuum light source with center wavelenght ?≈820nm and spectral bandwidth ~120nm. A CMOS camera was used to capture the spot image at different distances.

Results and conclusion. For the case of f1 and f2 the axicon lens keep the longest depth of focus but at the same time the lowest intensity of the three methods while axilens has the highest intensity and good DOF, for the case f2 the multiplexe lens don't codify well the beam. In conclusion the axilens presents the most convenient profile because have highest intensity and good depth of focus.

8936-36, Session 8

Demonstration of a pulsed Raman fiber laser for tissue marking and integration with an OFDI system sharing a single-mode fiber optic probe

Hyoung Won Baac, Martin L. Villiger, William Lo, Brett E. Bouma, Harvard Medical School (United States)

Clinically, tissue biopsy remains a routine procedure for the diagnosis of pathological conditions, but it is subject to sampling errors leading to diagnostic uncertainty. Laser marking on tissue can provide an accurate means for the localization of suspicious lesions under the guidance of optical frequency domain imaging (OFDI). While several fiber lasers have been used previously, their performance was substantially limited by the laser energy available for coagulation, inefficient thermal energy deposition (e.g. continuous-wave laser irradiation), and wavelength compatibility with an OFDI system. These slowed down the laser-marking process and caused potential motion artifacts under physiological conditions. Therefore, the use of higher laser energy is required. However, it is challenging to develop such a system that simultaneously shares a single-mode fiber with OFDI.

We present a high-energy pulsed fiber Raman laser (>20 mJ/pulse at 1.44-?m wavelength) that can greatly improve overall marking efficiencies in terms of speed and thermal energy deposition. We demonstrate that this pulse energy far exceeds theoretical estimation (~1 mJ) for single-pulsed tissue coagulation in the focal spot. Furthermore, we introduce our efforts to integrate the fiber Raman laser with our OFDI system. First, we investigate optimal marking conditions by performing tissue coagulation in situ under OFDI, using a coaxial alignment of both marking and imaging laser beams on linear motion stages. These parameters will be used to design an integrated system on a rotary stage in which sufficient laser energy should be provided to overcome insertion losses due to optical components, while excluding possible thermal damage.

8936-37, Session 8

Advancements on galvanometer scanners for high-end applications

Virgil-Florin Duma, Aurel Vlaicu Univ. of Arad (Romania); Jannick

P. Rolland, Univ. of Rochester (United States)

Galvanometer-based scanners (GSs) are the most used device for lateral scanning. Their applications range from commercial and industrial to biomedical imaging. They are used mostly for 2-D scanning (in dual axis double GSs systems), but also for 1-D or 3-D scanning (the latter by example with GSs in combination with Risley prisms). This paper presents an overview of our contributions in the field of GSs with regard to the requirements of their most challenging applications. The presentations is structured on the following parts: (i) study of optimal scanning functions – to produce the maximum possible duty cycle; (ii) experimental investigations of the GS input signals to determine the scanning regimes that produce the minimum image artifacts, by example in Optical Coherence Tomography (OCT); (iii) command functions of GSs, to achieve a trade-off between performance criteria like duty cycle and voltage regimes of the device; (iv) control solutions of the devices – from classical to advanced – to obtain the highest possible precision or the fastest possible response of the scanner. An application in OCT is presented to demonstrate the influence of the scanning regimes on the quality of the images.

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8936-38, Session PSun

Effect of noise levels of an edge image on determining the presampled modulation transfer function

Wenbo WAN, Feng Gao, Tianjin university (China); Huijuan Zhao, Lixin Zhang, Zhongxing Zhou, Tianjin Univ. (China)

The modulation transfer function (MTF) is widely used to describe the spatial resolution of x-ray imaging systems. Extensive works have been conducted to achieve accurate and precise measurement of MTF by using a slanted edge test device. The noise level of the slanted edge image is an important factor influencing the accuracy of MTF measurement. Thus in this work, a comparison study was made on the MTF measurement results obtained by using different curve fitting algorithms for ESF determination when analyzing the same image data with different noise levels. The results indicated that the averaged MTF measurement errors got increased with the decrease of the signal-to-noise ratio of the slanted edge images for all of the ESF processing algorithms. But for the same noisy slanted edge image, monotonic fitting algorithm outperformed Gaussian smoothing method or moving polynominal fitting method on MTF measurement.

8936-39, Session PSun

3D papillary image capturing by the stereo fundus camera system for clinical diagnosis on retina and optic nerve

Danilo A. Motta, Wavetek Technologies Industry Ltd. (Brazil); Andre Serillo, Wavetek Technologies Industry LTDA (Brazil); Luciana de Matos, Fatima M. M. Yasuoka, Univ. de São Paulo (Brazil) and Wavetek Technologies Industry Ltd. (Brazil); Vanderlei Salvador Bagnato, Univ. de São Paulo (Brazil); Luis Albert V. Carvalho, Wavetek Technologies Industry Ltd. (Brazil) and Univ. de São Paulo (Brazil)

Glaucoma is the second main cause of the blindness in the world and there is a tendency to increase this number due to the lifetime expectation raise of the population. Glaucoma is related to the eye conditions, which leads the damage to the optic nerve. This nerve carries visual information from eye to brain, then, if it has damage, it compromises the visual quality of the patient. In the majority cases the damage of the optic nerve is irreversible and it happens due to increase of intraocular pressure. One of main challenge for the diagnosis is to find out this disease, because any symptoms are not present in the initial stage. When is detected, it is already in the advanced stage. Currently the evaluation of the optic disc is made by sophisticated fundus camera, which is inaccessible for the majority of Brazilian population. The purpose of this project is to develop a specific fundus camera without fluorescein angiography and red-free system to accomplish 3D image of optic disc region. The innovation is the new simplified design of a stereo-optical-system, in order to make capable the 3D image capture and in the same time quantitative measurements of excavation and topography of optic nerve; something the traditional fundus cameras do not do. The dedicated hardware and software is developed for this ophthalmic instrument, in order to permit quick capture and print of high resolution 3D image and videos of optic disc region (20degree field of view) in the mydriatic and no-mydriatic mode.

8936-40, Session PSun

Extracting optical properties of turbid media using radially and spectrally resolved diffuse reflectance

Jonathan Malsan, Northeastern Univ. (United States) and Radiation Monitoring Devices, Inc. (United States); Rajan Gurjar, David E. Wolf, Karthik Vishwanath, Radiation Monitoring Devices, Inc. (United States)

The determination of tissue optical coefficients (absorption (μa) and scattering (μs')) is critical for many light-based biomedical sensing applications. In order to translate experimental measurements of diffuse reflectance or transmittance into the absorption and scattering properties, frequently, it is necessary to use reference tissue phantoms as they provide a control set of optical properties for comparison. Here we explore different methods to extract the optical coefficients of solid tissue phantoms using radially resolved diffuse reflectance (RRDR) and spectrally-resolved diffuse reflectance (SRDR).

Experimental measurements of RRDR were obtained from semi-infinite solid phantoms. For these measurements, we affixed a source fiber to the surface of the phantom and moved a detection fiber using a micrometer stage to span source-detector separations of 1-13mm in 0.63mm increments. Reflectance spectra (between 400-850 nm) were obtained at each separation. These data were processed to produce RRDR for each integer wavelength and fit to multiple theoretical models to extract μa and μs' coefficients.

The theoretical RRDR based models will produce a set of optical coefficients for each phantom, which will be used as reference phantoms in an inverse Monte Carlo (iMC) SRDR algorithm to derive optical coefficients of liquid phantoms. These liquid phantoms will be prepared by mixing known volumes of hemoglobin, polystyrene microspheres and water, thus allowing determination of its optical coefficients. For each set of RRDR-derived optical coefficients used as iMC reference phantoms, we will compare the iMC extracted optical properties of the liquid phantoms against their known values, establishing the validity of the RRDR-derived coefficients.

8936-41, Session PSun

Characterization and modeling of point spread function in push-broom hyperspectral imaging systems for spectral and spatial resolution enhancement

Jurij Jemec, Miran Bürmen, Franjo Pernu?, Bo?tjan Likar, Univ. of Ljubljana (Slovenia)

Hyperspectral imaging systems (HIS) are becoming widely used in numerous biomedical applications such as cancer diagnosis and treatment, burn depth assessment, monitoring tissue oxygenation, blood glucose and other blood metabolites or drugs. Among the variety of HIS, push-broom systems stand out for the good signal to noise ratio and short acquisition time. Push-broom systems consist of three main units: the front lens, the dispersing PGP (Prism-Grating-Prism) spectrograph and a camera. The PGP spectrograph ideally disperses the spectral and spatial information in two orthogonal directions preferably aligned with the columns and rows of the imaging sensor. Due to the imperfections in the camera lens and in particular the optical components of PGP, wavelength dependent spectral and spatial distortions along with spatial and spectral blur are introduced in the recorded image. In this study we propose and evaluate a method for spectral and spatial resolution enhancement of push-broom HIS. First, the spatially and spectrally dependent point-spread function (PSF) was characterized by measuring the response of the system to several reference objects. The relevant variability of the PSF over the imaging plane was captured by a global parametric model. Finally, the estimated PSF was used to enhance the spectral and spatial resolution of the system. The resolution enhancements were objectively assessed by observing the change in full width at half-maximum of spectral lines and the system response to a 1951 USAF resolution target. The results show that significant spectral and spatial resolution enhancements can be obtained by the proposed method.

8936-42, Session PSun

Optical method to estimate the alcohol concentration by analyzing the human skin

Norma P. Puente, Griselda Quiroz-Campean, Univ. Autónoma de Nuevo León (Mexico); Aurora Espinoza-Valdez, Universidad de Guadalajara (Mexico)

In this paper we present a novel method for obtaining a measurement of the amount of alcohol concentration in human blood. The detection is based on the scattering of light (? = 543 nm) on human skin. The most important parameter in this work is the pore size on the skin, it is reported that the pore size is related to the hydration of the human body, and when alcohol is consumed it dehydrates the human body. In this paper we report the statistics of a group of people with different blood alcohol concentrations and real-time monitoring.

8937-1, Session 1

Multimodal stereoscopic optical intraoperative imaging

Vyacheslav Kalchenko, Weizmann Institute of Science (Israel); Igor Meglinski, Univ. of Otago (New Zealand); Yuri Kuznetsov, Alon Harmelin, Weizmann Institute of Science (Israel)

Nowadays it's a standard practice when small laboratory animals are used as models of humans or other species. The small animals involved in various studies associated with cancer, drugs delivery, toxicity screening, studying gene mutation, gene transcription, treatment efficiency assessment, as well as practical models for neurosurgeons. Last decade much effort has been spent on the development of advanced imaging technique that provides better navigation capabilities during microsurgery. We present a stereoscopic multimodal imaging approach that combines real time fluorescence imaging (FI) and fast laser speckle imaging (FLSI) modalities. FI utilizes intra-dermal administration of fluorescent marker that labeled of lymphatic system followed by standard visible light fluorescence image acquisition. FLSI uses the near-infrared (NIR) coherent light source and an additional CCD camera incorporated in the same optical system. To provide fast imaging in real-time a special algorithm has been developed. We demonstrated an FI guided microsurgical manipulation with popliteal and inguinal lymph nodes (LN) of mice, assisted by simultaneous label free visualization of LN blood vessels using FLSI. We conclude that combined FI-FLSI approach provides better navigation capabilities during microsurgery and allows avoiding unnecessary damage of blood vessels.

8937-2, Session 1

A surgical navigation system for noncontact diffuse optical tomography and intraoperative cone-beam CT

Michael J. Daly, Univ. of Toronto (Canada); Jonathan C. Irish, Brian C. Wilson, David A. Jaffray, Princess Margaret Cancer Ctr. (Canada)

A freehand, non-contact diffuse optical tomography (DOT) system has been developed for multimodal imaging with intraoperative cone-beam CT (CBCT) during minimally-invasive cancer surgery. This translational research system is under investigation for clinical applications in head-and-neck surgery including oral cavity tumour resection, lymph node mapping, and free-flap perforator assessment. The DOT system is configured for fluorescence imaging with indocyanine green (ICG) using a collimated 760 nm laser diode (Thor Labs) and a near infrared CCD camera (PCO Pixelfly-USB). Depending on the intended surgical application, the camera is coupled to either a rigid endoscope (Karl Storz 10-mm) or a compact lens (Edmund Optics 25-mm). A flat-panel CBCT C-Arm (Siemens Healthcare) acquires low-dose 3D images with sub-mm spatial resolution. A 3D mesh is extracted from CBCT for finite element DOT implementation in NIRFAST (Dartmouth College), with the capability for soft/hard imaging priors (e.g., segmented lymph nodes). A stereoscopic optical camera (NDI Polaris) provides real-time 6D localization of reflective spheres mounted to the laser and camera. Camera calibration combined with tracking data is used to estimate intrinsic (focal length, principal point, non-linear distortion) and extrinsic (translation, rotation) lens parameters. Source/detector boundary data is computed from the tracked laser/camera positions using radiometry models. Target registration errors (TRE) between real and projected boundary points are <2 mm for typical acquisition geometries. Pre-clinical studies using tissue phantoms and small animals are presented to characterize 3D imaging performance as a function of inclusion size, depth, and concentration.

8937-3, Session 1

Multimodal confocal mosaics enable high sensitivity and specificity in screening of in situ squamous cell carcinoma

Anna Bar, Nicholas Snavely, Steven Jacques, Oregon Health & Science Univ. (United States); Daniel S. Gareau, The Rockefeller Univ. (United States)

Screening cancer in excision margins with confocal microscopy is under investigation as a means to potentially save time and cost over the gold standard histopathology (H&E). However, diagnostic accuracy requires sufficient contrast in a growing set of tumor types. Reflectance mode enables imaging of structural details due to microscopic refractive index variation. Nuclear contrast with acridine orange fluorescence provides enhanced diagnostic value, but fails for early stage, in situ squamous cell carcinoma, where the cytoplasm is important to visualize. Combination of three modes detects squamous cell carcinoma (SCC) in situ. Accurate screening of SCC requires eosin fluorescence, reflectance and acridine orange fluorescence to enable contrast for cytoplasm, collagen and nuclei respectively. Combining these signals replicates H&E well and may enable rapid clinical translation.

8937-4, Session 1

Single-snapshot widefield optical properties imaging

Jean Vervandier, Sylvain Gioux, Beth Israel Deaconess Medical Ctr. (United States)

Current methods for imaging optical properties over wide fields of view (> 100 cm2) typically involve either raster-scanning a point source and processing in the temporal or spatial domain, or more recently imaging of spatially modulated planar illuminations and processing in the spatial frequency domain. While the latter is being faster than raster scanning a point source, current state-of-the-art requires 6 images to be acquired for each optical properties map processed. The need for several images to be acquired remains a strong limitation for real-time use in clinical practice, in particular during applications where movement artifacts are present.

In this study we present a novel method involving the acquisition of a single image to extract optical properties over a wide field of view. More particularly, this method focuses on degrading spatial resolution to the benefit of temporal resolution. It relies on processing in the spatial frequency domain, using a single image containing multiple frequencies to measure both absorption and reduced scattering. This approach has been validated against current state-of-the-art in SFD imaging on homogenous silicone phantoms mimicking living tissues optical properties and in vivo onto Yorkshire pigs during vascular occlusion experiments. Overall, this method performs similarly to the state-of-the-art, with minor spatial resolution degradation and allows for single snapshot imaging of optical properties over wide fields of view.

8937-5, Session 1

A simultaneous multimodal imaging system for tissue functional parameters

Wenqi Ren, Univ. of Science and Technology of China (China); Zhiwu Zhang, University of Science and Technology of China (China); Qiang Wu, Shiwu Zhang, Univ. of Science and

Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

Simultaneous assessment of functional and structural characteristics will facilitate quantitative and three-dimensional diagnosis in many clinical applications such as wound healing treatment. However, many existing clinical practices and imaging systems are subjective, two-dimensional, or sequential for multimodal data collection. To overcome these limitations, we developed a multimodal and multiview imaging system for non-invasive, quasi-concurrent, quantitative, and three-dimensional imaging of cutaneous tissue oxygenation, blood perfusion and structural characteristics. The imaging system integrated hyperspectral, laser speckle, and multiview imaging technologies into one setup. An AOTF tunable light source and a 785nm laser device were connected to a ring-shaped light pipe for illumination via a bifurcated fiber bundle, a square mirror was placed between a high-resolution CCD camera and the ring illuminator for multiview reflective imaging. A Labview interface was developed for equipment control, synchronization, and image acquisition. Advanced algorithms were developed for accurate reconstruction of tissue oxygenation, blood perfusion, and 3D topography. The system was under quantitative validation tests in a skin-simulating phantom test, an in vivo post-occlusion reactive hyperemia (PORH) procedure, and an ongoing clinical trial of wound healing process monitoring last for two weeks. The experiments results were compared and calibrated by moor (Moor Instruments Inc., Devon, UK) tissue oxygenation monitor and laser Doppler monitor. In this study, we have not only setup a multimodal and multiview imaging system for cutaneous tissue functional and structural characteristics but also demonstrated its potential for wound healing assessment in clinical practice.

8937-6, Session 1

TBD *(Invited Paper)*

No Abstract Available

8937-7, Session 2

Multimodal confocal microscopy based on a double-clad fiber coupler

Etienne De Montigny, Wendy-Julie Madore, Ecole Polytechnique de Montréal (Canada); Mathias Strupler, Sainte-Justine Hospital Research Ctr. (Canada); Amber M. Beckley, Ecole Polytechnique de Montréal (Canada); Frédéric Leblond, Ecole Polytechnique de Montréal (Canada) and Ctr. Hospitalier de l'Univ. de Montreal Research Ctr. (Canada); Nicolas Godbout, Ecole Polytechnique de Montréal (Canada); Caroline Boudoux, Ecole Polytechnique de Montréal (Canada) and Sainte-Justine Hospital Research Ctr. (Canada)

Combining coherent modalities (such as optical coherence tomography (OCT) or reflectance confocal microscopy (RCM)) with efficient detection of incoherent weak signals (such as fluorescence or Raman) may be performed through double-clad fibers (DCF). To separate the signal traveling into the core from the multimode signal in the cladding, double-clad fiber couplers (DCFC) were developed. These couplers are typically fabricated using commercial double-clad fibers with a cut-off wavelength of 1250nm, which reduces the applicability of this technology to long wavelengths and severely limits the selection of fluorophores.

We present a novel double-clad fiber coupler for combined RCM and fluorescence imaging at 800nm using a custom double-clad fiber (5:25:125µm, cut-off wavelength: 610nm). The inner-cladding-to-core ratio of the DCF was chosen to preserve optical sectioning while reducing speckle noise in RCM. This ratio also allows for a 25 fold increase in fluorescence signal collection compared to single mode fiber. We achieved >90% transmission through the core and >70% extraction of inner cladding signal in an achromatic fashion from 760 to 800nm. We demonstrate the use of this DCFC in a combined reflectance and fluorescence confocal microscope based on spectral encoding. Co-registered reflectance and fluorescence images of 512x512 pixels were simultaneously acquired at 20 frames per second. This all-fiber device provides a gain in signal compared to a free-space beamsplitter/dichroic mirror approach and is robust enough to be integrated into a clinical endomicroscopy system.

8937-8, Session 2

Resonant hyperspectral CARS and FWM microscopy of in-vivo carotenoid accumulation in H. Pluvialis

Aaron D. Slepkov, Trent Univ. (Canada); Aaron M. Barlow, National Research Council Canada (Canada) and Univ. of Ottawa (Canada); Andrew Ridsdale, National Research Council Canada (Canada); Joel T. Tabarangao, Trent Univ. (Canada); Albert Stolow, National Research Council Canada (Canada) and Univ. of Ottawa (Canada)

We use a spectral-focussing-based femtosecond nonlinear microscopy platform to implement multimodal imaging comprising coherent anti-Stokes Raman scattering (CARS), four-wave-mixing (FWM), and two-photon excitation fluorescence (TPEF) modalities. Due to resonant enhancement, CARS and FWM signals from astaxanthin within microalgae cysts are dominant at our pump wavelengths. This enables rapid nonresonant-background (NRB)-free spectral characterization at concentrations as low as or 1.5 mM, without the need for phase-retrieval or frequency-modulation procedures. In the fingerprint region, the strong in vivo carotenoid signals are more than 300X stronger than those from a bulk diamond reference. In the CH-stretch region, we observe signals which are vibrationally nonresonant. These represent electronically-resonant FWM signals which are sufficiently strong for high-contrast imaging of carotenoids with an order of magnitude lower laser power than we typically use for live-cell imaging. In both vibrational frequency regions, concurrent TPEF imaging maps the spatially-distinct chlorophyll-rich regions. This research has direct applications to imaging the bioaccumulation of carotenoids in H. Pluvialis—currently a >$300M industry—as well as for carotenoid-based drug monitoring and delivery. Finally, resonant CARS imaging is important within coherent Raman microscopy techniques, particularly when implemented at third order which avoids the direct linear absorption of either the pump or Stokes input beams.

8937-9, Session 2

Design, fabrication, and characterization of a compound lens for simultaneous optical coherence tomography and confocal microscopy

Mathias Strupler, Ecole Polytechnique de Montréal (Canada) and Sainte-Justine Hospital Research Ctr. (Canada); Etienne De Montigny, Amber M. Beckley, Ecole Polytechnique de Montréal (Canada); Caroline Boudoux, Ecole Polytechnique de Montréal (Canada) and Sainte-Justine Hospital Research Ctr. (Canada)

In open surgeries, the ability to localize and characterize small structures (such as glands, nerves and lymph nodes) is a key factor of success. Currently this task is performed by using frozen sections following excision of part of the structure of interest. To accelerate and simplify this procedure, we wish to provide surgeons with a handheld tool combining two complementary non-invasive modalities: optical coherence tomography (OCT) having a large field of view (FOV) and confocal microscopy (CM) having a high resolution. In order to perform

imaging with OCT and CM simultaneously, we created a lens that can accommodate the optical requirements for both modalities while fitting in a 1 cm diameter handheld device.

We designed a cemented triplet consisting of custom acrylic and polystyrene lenses. The triplet handles two wavelength bands: one at 1250-1350 nm for OCT and another at 770-870 nm for CM. It is image telecentric and achromatized for the CM wavelength band. We then fabricated this triplet using a diamond turning lathe, using 1-inch diameter plastic blanks to machine the lenses. It allowed us to include centering and alignment features that could be removed after the lens assembly to obtain the 8-mm diameter objective lens.

We finally inserted the lens in an optical setup that combines OCT and CM. In CM, the system can resolve more than 228 cycles per mm and has a field of view larger than 0.5-mm. In OCT, the lens allows for a FOV >2.5mm and a numerical aperture of 0.03.

8937-10, Session 2

In-vivo widefield imaging of a fluorescent deoxy-glucose bioprobe: guiding multiphoton microscopy in oral epithelial neoplasia

Rahul Pal, Jinping Yang, Gracie Vargas, The Univ. of Texas Medical Branch (United States)

Multiphoton autofluorescence microscopy (MPAM) and second harmonic generation microscopy (SHGM) have shown the potential to identify optical and spectroscopic features which are directly related to the organization and microstructure of biological specimens that may be differentiated between normal and neoplastic tissues, however limited to a small field of view. A method that provides large area indication of suspicious regions would help in guiding MPAM-SHGM image sites given the inability to screen a full tissue. We have utilized a fluorescent glucose analog 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) for Wide-field assessment, guiding towards areas of abnormally high glucose uptake that indicates regions of dysplasia/cancer, followed by high resolution MPAM-SHGM to assess morphological and spectral features.

A hamster model of oral carcinogenesis was used in this study which involved topical application of 9,10-dimethyl-1,2-benzanthracene (DMBA). 1mg of 2-NBDG in PBS was delivered by intravenous injection and the entire cheek pouch was imaged in-vivo in a wide-field setup using appropriate excitation and emission filters. Wide-field images were analyzed based on 2-NBDG fluorescence intensity. Areas of high fluorescence and controls were selected for in-vivo microscopic analysis via MPAM-SHGM. Imaged sites were biopsied and processed, with hematoxylin stained sections graded by a pathologist.

Our results show the feasibility of this combinatorial approach of large area assessment and subsurface microscopic evaluation of neoplastic oral mucosa. Wide-field fluorescence revealed areas of high glucose uptake and subsequent microscopic analysis indicated preneoplastic or neoplastic features. We have demonstrated a relationship between gross metabolic changes and noninvasively measurable optical signals during neoplastic development.

8937-11, Session 2

In-vivo measurements of oxy- and deoxyhemoglobin levels in breast cancer xenografts in a mammary window chamber model

Hui Min Leung, College of Optical Sciences, The Univ. of Arizona (United States); Rachel Schafer, Arthur F. Gmitro, The Univ. of Arizona (United States)

Cancer cells are characterized by adaptive features that allow them to evade apoptosis and proliferate in an unchecked manner in the host tissue. Therapeutic strategies often involve targeting those adaptive molecular pathways leading to downstream effects such as changes in perfusion, metabolic rate, and/or oxygen utilization in the malignant tissue. Such surrogate biomarkers can be used to monitor therapeutic response, optimize treatment protocols, or assist in development of new therapeutic approaches. In this study, we present an optical methodology to make in vivo measurements of the levels of oxy- and deoxyhemoglobin as a surrogate biomarker in breast cancer xenografts within a mouse mammary window chamber (MWC) model. By using multi-spectral measurements of the reflectance off the tissue under the coverslip of the window chamber, we obtain high-resolution maps of the oxy- and deoxyhemoglobin levels in the tissue, which allow continuous monitoring of the level of blood oxygenation during tumor growth and following treatment. The MWC, which was designed and fabricated in-house, is compatible with multiple imaging modalities such as MRI and high resolution intravital microscopy providing the capability for cross validation of oxygenation measurements on multiple imaging platforms.

8937-12, Session 3

A parallel framework for simultaneous FNIRS/FMRI fusion

Zhen Yuan, Univ. of Florida (United States) and Univ. of Macau (China)

Concurrent fNIRS/fMRI recordings represent multiple, simultaneously active, regionally overlapping neural hemodynamic responses. In this study, we propose a novel parallel framework for the spatiotemporal fNIRS/fMRI fusion to address the issues due to the overlapping nature of these responses. The developed fusion techniques employ the Independent Component Analysis to recover the time courses and spatial mapping components from fNIRS and fMRI separately. Then the correlated components from each imaging modality are combined concurrently in the spatial and temporal domain for fMRI-guided fNIRS and fNIRS aided fMRI.

8937-13, Session 3

MRI-guided optical spectroscopy of human breast cancer increases information content of clinical DCE-MRI

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Image guided optical spectroscopy has been developed as a non-invasive tool to compliment contrast MR imaging of breast cancer with functional maps of tissue physiology, prior to biopsy. In optimizing this multimodality imaging technique, we hope to be able to aid healthcare decisions while providing more information to clinical MRI exams. In this study, a 9 wavelength hybrid frequency domain and continuous wave NIRS system is used to image pre-surgical breast cancer patients. Images of total hemoglobin, oxygen saturation, water and lipid percentage, and scattering parameters are reconstructed using a priori information from the MRI scan. We present results from an ongoing clinical study (target 60 patients) where this technology is used to try to separate malignant lesions from benign, prior to biopsy. 48 patients have been enrolled as of 8/22. Imaging results of suspicious regions are compared with histo-pathological analysis of surgical samples from the same regions. Early results suggest the ability to separate malignant

lesions from benign masses based on total hemoglobin with statistical significance (mean tumor/background contrast 1.53x/0.93x). The results of this complete study will be presented in detail, analyzing optical chromophores and scattering information vs. tissue types as well as vs. MRI-recovered parameters. We also present discussion of how to best incorporate this technology into clinical workflow to increase the amount of prognostic information at the time of MRI for breast cancer patients. This combination provides complex anatomical and molecular information that may decrease the number of unnecessary biopsies thereby improving patient care.

8937-14, Session 3

Dual-mode dynamic imaging of breast cancer

Shiwu Zhang, Min Xu, Junnan Zhang, Univ. of Science and Technology of China (China); Qingping Tong, The 105th PLA Hospital (China); Pengfei Shao, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

Breast cancer remains the leading cause of newly diagnosed cases of cancer among women nowadays. One commonly used technique for breast cancer screening is ultrasonography. The technique reveals tumor structural characteristics and blood flow. Elastic ultrasonography differentiates the deformation of different tissue compartments in response to an external compression force. However, this method has low specificity and is not reliable. Near infrared oximetry detects tissue functional parameters such as oxygenation and hemoglobin concentration. However, the technique has relatively low spatial resolution. Considering the complimentary advantages of ultrasonography and tissue oximetry, we have integrated a near infrared tissue oximeter, a clinical ultrasound probe, and multiple pressure sensors in a single handheld piece for noninvasive detection of pressure-induced structural and functional dynamics of suspicious breast lesions. The handheld piece is designed based on the existing detection elements and fabricated by a rapid prototyping technique. It holds 8 source fibers, 1 detecting fiber connected to an OxiplexTS tissue spectrophotometer, 4 miniature load cells, and a Hitachi L74M ultrasound probe. During the experiment, the handheld piece is compressed against the breast tissue at a consistent amplitude and frequency. Tissue dynamic response to the cyclic compression load is recorded by the tissue oximeter and the ultrasound transducer simultaneously. The collected optical and ultrasound data are further analyzed to reveal the dynamic characteristics of suspicious breast lesion in terms of tissue oxygenation, hemoglobin, blood flow, and deformation. The integrated system has been assembled and will be deployed to the clinical site for a clinical trial.

8937-15, Session 3

A true multimodality approach for high resolution optical imaging: photo-magnetic imaging

Alex T. Luk, Univ. of California, Irvine (United States)

Multi-modality imaging leverages the competitive advantages of different imaging systems to improve the overall resolution and quantitative accuracy. Our new technique, Photo-Magnetic Imaging (PMI) is one of these true multi-modality imaging approaches, which can provide quantitative optical absorption maps at MRI spatial resolution. PMI uses laser light to illuminate the tissue and elevate the temperature while utilize MR thermometry to measure the laser induced temperature variation with high spatial resolution. The high resolution temperature maps are later converted to tissue absorption maps by a finite element based inverse solver that utilize modeling of photon migration and heat diffusion in tissue. Previously, we have demonstrated the feasibility of PMI with phantom studies. Recently, we managed to reduced the laser power under ANSI limits for maximum skin exposure so we well positioned ourselves for in vivo imaging. Currently we are expanding our system for multi-wavelength imaging by adding multiple lasers illuminating tissue at different wavelengths. This will allow us not only to resolve spatial distribution of tissue chromophores but also exogenous contrast agents. In this abstract, we will present in vivo study results using fisher rats bearing R3220 AC breast cancer tumor models. For these studies, we use gold nanorods as well as Indocyanine green (ICG) as contrast agents. Although we can detect presence of contrast agents using single-wavelength PMI system, our aim is to utilize multi-wavelength for rendering quantitatively correct contrast agent distribution. Since PMI has a high translational potential, we are also developing a PMI breast imaging interface. We will also present development of this clinical PMI system for breast cancer.

8937-16, Session 3

Validation of temperature-modulated fluorescence tomography in vivo

Tiffany C. Kwong, Univ. of California, Irvine (United States)

To overcome the strong scattering in biological tissue that has long afflicted fluorescence tomography, we have developed a novel technique, “temperature-modulated fluorescence tomography” (TM-FT) to combine the sensitivity of fluorescence imaging with focused ultrasound resolution. TM-FT relies on two key elements: temperature sensitive ICG loaded pluronic nanocapsules we termed ThermoDots and high intensity focused ultrasound (HIFU). TM-FT localizes the position of the fluorescent ThermoDots by irradiating and scanning a HIFU beam across the tissue while conventional fluorescence tomography measurements are acquired. The HIFU beam produces a local hot spot, in which the temperature suddenly increases changing the quantum efficiency of the ThermoDots. The small size of the focal spot (~1 mm) up to a depth of 6 cm, allows imaging the distribution of these temperature sensitive agents with not only high spatial resolution but also high quantitative accuracy in deep tissue using a proper image reconstruction algorithm. Previously we have demonstrated this technique with a phantom study with ThermoDots sensitive in the 20-25°C range. We recently optimized the ThermoDots for physiological temperatures. In this work, we will demonstrate the first animal study results for TMFT using our new animal interface and ThermoDots optimized for in vivo imaging.

8937-17, Session 3

Multiplexed small animal fluorescence imaging using joint fluorescence spectral and lifetime information

Niksa Valim, Mark Niedre, Northeastern Univ. (United States)

Fluorescence imaging is a powerful method for the study of cellular and molecular events in vivo. However, the overlap of the emission spectra of red and near infrared fluorophores combined with the relatively narrow optical “diagnostic window” makes imaging and separation of multiple (3 or more) targets in deep tissues in small animals extremely challenging. In this work, we addressed this problem by jointly acquiring and analyzing both spectral and lifetime information of fluorophores.

We measured multi-spectral and temporal fluorescence data sets in phantoms and in vivo using a pulsed supercontinuum excitation laser and a fiber-coupled spectrograph with a 16-channel photomultiplier tube (PMT) array. The instrument was operated in time correlated photon counting mode, so that in combination we could measure transmitted time-resolved photons through the sample with 13 nm spectral and 8 ps temporal resolution. We used five fluorophores with emission maxima in the red and near infrared range (700-900 nm) and with lifetimes in the range of 1.5-3.2 ns. We first embedded combinations of the fluorophores in a solid tissue mimicking diffusing phantom with 1.5 cm pathlength

and then scanned the object with an x-y translation stage. The signal components were then analyzed and de-mixed using a set of three of chemometric algorithms. Our results show that joint use of spectral and temporal data decreases the mean error in the estimated concentration by a factor of 3-6 and 3.5-6.5 times compared to purely spectral data or temporal data, respectively. In vivo validation of the technique is in progress.

8937-18, Session 4

Detection, enumeration, and tracking of extremely rare circulating cells in vivo with diffuse fluorescent light

Mark J. Niedre, Noah Pestana, Stacey Markovic, Vivian E. Pera, Northeastern Univ. (United States)

In vivo flow cytometry (IVFC) represents an emerging set of technologies that allow non-invasive detection and enumeration of circulating cells in the bloodstream in small animals. IVFC has many applications in biomedical research including cancer metastasis, immunology, organ transplants and stem cell therapeutics. There is a continuing need for new IVFC instruments with extremely high detection sensitivity, for example, in the study of early stage metastasis or minimal residual disease.

In this presentation we describe the results of several of our recent studies focused on the detection and enumeration of very rare circulating cell populations (<100 cells per mL of peripheral blood). First, we developed an instrument that utilizes interrogation of relatively large tissue and blood volumes with diffuse photons in a small animal limb (e.g. hindleg or tail). The instrument employs multi-spectral, multi-optode detection of emitted diffuse fluorescent light using, i) efficient optical collection, ii) high sensitivity electronics, and iii) suppression of movement artifacts. We demonstrate that in combination this allows detection of individual circulating cells at concentrations close to 1 cell/mL in vivo, and tomographic imaging of the cell in the limb cross- section with an accuracy of ~100 ?m. Second, we used a wide-field, high-gain fluorescence imager to interrogate large tissue regions (~1 x 1 cm2 section of the mouse ear). We developed an automated computer vision algorithm to identify, count and track the paths of individual labeled circulating cells from noisy image sequences at concentrations in the range of 10 cells / mL of peripheral blood.

8937-19, Session 4

One-step microencapulation of nanoparticles and perfluorocarbon in microbubbles for potential application in controlled activation

Guangbin Li, Ting Si, Xisheng Luo, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

Recently, metallic nanoparticles and activatable agents, such as gold nanoparticles, silver nanoparticles, and perfluorocarbon, have attracted many attentions by researchers in the field of biomedical optical imaging. These agents can be encapsulated in microbubbles or microcapsules in order to achieve multimodal imaging contrast and controllable activation for multifunctional applications such as photoacoustic imaging and ultrasound/light mediated drug delivery. However, commonly used microencapsulation processes have a low encapsulation efficiency and cannot support further application of these novel agents. To increase the encapsulation efficiency, a coaxial electro-flow focusing (CEFF) process that combines coaxial electrospray with flow focusing has been developed. The process can be characterized as a coaxial liquid jet of nanoparticles and perfluorcarbon in the core of a high-speed coflowing gas stream under an axial electric field. Microcapsules can be obtained after the breakup of the coaxial liquid jet. On the experimental side, an experimental system consisting of a customized coaxial needle, a gas container, two high-voltage power supplies, a dual-channel syringe pump, a collection reservoir and process monitoring accessories has been built up. Different flow modes have also been obtained. On the theoretical side, the instability of the coaxial jet under different conditions has been studied systemically based on the classical normal mode method. The effects of main process parameters, such as electric field intensity, pressure difference, flow rates of inner and outer liquids and interfacial tension on the breakup of the jet have been evaluated. Finally, microcapsules that encapsulate perfluorocarbon and nanoparticles with nearly 100% loading rate and good morphology have been obtained. The present research provides quantitative control and optimization of the CEFF process for the fabrication of the multifunctional activatible microbubbles.

8937-20, Session 4

Single snapshot RGB multispectral imaging at fixed wavelengths: proof of concept

Janis Spigulis, Liene Elste, Univ. of Latvia (Latvia)

A concept of single snapshot multispectral imaging by standard RGB image sensors under spectrally-selective illumination comprising a fixed number of narrow spectral lines is discussed. The limiting conditions, RGB band spectral crosstalk corrections and potential applications for parametric mapping of skin will be discussed, along with the preliminary results of the proof-of-concept measurements.

8937-21, Session 4

Microencapsulation of multiple components by compound-fluidic electro-flow focusing

Chuansheng Yin, Ting Si, Peng Gao, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

Microparticles with multiple compartments inside are necessarily required in various applications including multicomponent drug delivery, microreactor, multimodal imaging and image-guided therapy. The most commonly used emulsion is unable to fabricate such microcapsules, and some other techniques including microfluidic channel, coaxial electrospray and flow focusing mainly focus on producing simple core-shell structure of microparticles. Here we report a compound-fluidic electro-flow focusing (CFEFF) process that could one-step envelope drugs and imaging agents into a single microcapsule without contact. In this method, the hierarchical compound needle is assembled by embedding two thin needles separately into a relatively large needle. The CFEFF process can be recognized as a combination of coaxial electrospray and flow focusing, in which a cone-jet configuration with two compartments inside can be formed in the core of a high-speed coflowing gas stream under an axial electric field. Eventually microcapsules can be obtained after the breakup of the liquid jet under both electric and hydrodynamic forces because of flow instability. In this work, an experimental system consisting of a hierarchical compound needle, a gas container, two high-voltage power supplies, three syringe pumps, a collection reservoir and process monitoring accessories is developed. The characteristics of the multicomponent cone-jet configurations are explored and different flow modes are identified. The effects of various process parameters on the morphology and size distribution of the microcapsules are further studied. The present research provides a quantitative guidance to optimize the CFEFF process for practice.

8937-22, Session 4

A fast and effective reconstruction method for fluorescence molecular tomography based on sparsity adaptive subspace pursuit

Jinzuo Ye, Chongwei Chi, Yu An, Han Xu, Shuang Zhang, Institute of Automation (China); Xin Yang, Institute of Automation, Chinese Academy of Sciences (China); Jie Tian, Institute of Automation (China)

Fluorescence molecular tomography (FMT) has many successful applications as a promising tomographic method for in vivo small animal imaging. However, FMT is usually an ill-posed problem since only the photon distribution over the body surface is measurable. The Lp-norm regularization is generally adopted to stabilize the solution, which can be regarded as a type of a priori information of the fluorescent probe bio-distribution. When FMT is used for the early detection of tumors, an important feature is the sparsity of the fluorescent sources because tumors are usually very small and sparse at this stage. Considering this, we propose a fast and effective method with L1-norm based on sparsity adaptive subspace pursuit to solve the FMT problem in this paper. This proposed method treats FMT problem with sparsity-promoting L1-norm as the typical pursuit problem. At each iteration, a sparsity factor that indicates the number of unknowns is estimated and updated adaptively. Then the algorithm seeks a small index set which indicates those atoms exhibiting highest correlation with the current residual, and updates the current supporting set by merging the newly selected index set. It can be regarded as a kind of sparse approximation reconstruction strategy. To evaluate this proposed method, we compare it to the iterated-shrinkage-based algorithm with L1-norm regularization in numerical experiments. Reconstruction results demonstrate that the proposed algorithm can obtain better results with greatly reduced number of iterations compared with the iterated-shrinkage-based algorithm, which makes it a practical and effective FMT reconstruction algorithm.

8937-23, Session PSun

IRF-calibrated Born normalization scheme for time-domain diffuse fluorescence tomography based on overlap time-gating

Feng Gao, Pengxi Liu, Wenbo Wan, JIao Li, Huijuan Zhao, Tianjin Univ. (China)

The full time-resolved (TR) methods of diffuse fluorescence tomography (DFT) are known to improve image resolution and accuracy significantly. However, these methods usually suffer from low practical efficacy due to the influence of the instrumental response function (IRF) and the tradeoff between the used data time-resolution and the required signal-to-noise ratio (SNR). We herein present a full TR approach that combines an IRF-calibrated full TR normalized Born approximation and an overlap-delaying time-gate scheme for attaining high SNR without sacrificing the TR information content. Phantom experiments demonstrate that the approach outperforms the traditional DFT methods in spatial resolution and reconstruction fidelity.

8937-24, Session PSun

Optimal arrangements of fiber optic probes to enhance the spatial resolution in depth for 3D reflectance diffuse optical tomography with time-resolved measurements performed with fast-gated single-photon avalanche diodes

Agathe Puszka, CEA-LETI (France); Laura Di Sieno, Alberto Dalla Mora, Antonio Pifferi, Davide Contini, Gianluca Boso, Alberto Tosi, Politecnico di Milano (Italy); Lionel Hervé, CEA-LETI-Minatec (France); Anne Planat-Chrétien, CEA-LETI (France); Anne Koenig, CEA (France); Jean-Marc Dinten, CEA-LETI-Minatec (France)

We investigate the performance of hand-held probes with short interfiber distances for time-resolved diffuse optical tomography and show how the quality of images is related to the arrangement of fibers. Probes with a width limited to a few centimeters could access intern organs like the prostate or facilitate the measurements on extern organs like the breast or the brain. We have recently shown on 2D tomographic images that time-resolved measurements with a large dynamic range obtained with fast-gated single-photon avalanche diodes could push forward the imaged depth range in a diffusive medium compared with conventional non-gated approaches. In this work, we confirm these performances with the first 3D tomographic images reconstructed with such a setup.

This setup includes a laser whose picosecond pulses are injected into an optical fiber. Light collected by a second fiber is sent to a single-photon avalanche diode (SPAD), connected to a time-correlated single-photon counting board. Operating the SPAD in fast-gating enables to explore the spatial resolution in a deeper range than with conventional techniques. The pair of source and detector is scanned at the surface of a phantom to simulate a multifiber probe with different interfiber distances (5, 10 and 15 mm). The imaged object consists in two punctual absorbing inclusions embedded a diffusive medium. All acquisitions were done for different inter-distances and depths of the inclusions. The data were processed by a 3D tomographic reconstruction algorithm based on the Mellin-Laplace transform. We conclude with suggestions for optimal arrangements of probes considering different clinical applications.

8937-25, Session PSun

Time-resolved measurements in diffuse reflectance: effects of the instrument response function of different detection systems on the depth sensitivity

Anne Planat-Chrétien, CEA-LETI (France); Michel Berger, CEA (France); Agathe Puszka, CEA-LETI (France); Lionel Hervé, Jean-Marc Dinten, CEA-LETI-Minatec (France)

We show the effect of the instrument response function (IRF) on reflectance time-resolved diffuse optical tomography. Using a femtosecond laser, we compare 3 detection modules: 2 single-photon detectors -a photomultiplier tube (PMT) and a hybrid PMT (respectively PMC-100 and HPM-100-50, Becker & Hickl GmbH)- coupled to time-correlated single-photon counting electronics (SPC-130, Becker & Hickl GmbH) and an intensified high rate imager (HRI, Kentech Instruments Ltd) coupled to a CCD camera (Orca-ER, Hamamatsu).

We analyze the depth sensitivity achieved for each detection module with an absorbing inclusion embedded in 2 turbid media (μs'=12 cm -1) of different absorption (μa=0.1 cm-1 or 0.2 cm-1) for 5, 10 and 15 mm interfiber distances. The Mellin-Laplace transform (MLT) is employed to process the time-resolved data. The analysis of the contrast on the different orders of MLT points out the well-known effects of the dynamic range but also the less studied impact of the tail of the IRF. It reveals

a loss of contrast on high orders of MLT for the deepest inclusions for IRFs featuring a tail and concludes on different maximal depths achieved for each IRF. Moreover, it defines the optimal number of MLT orders to analyze the data in each case.

For the maximal depth configurations, 2D planar scans were realized to reconstruct the 3D optical properties of the deeply buried inclusions for each detection system. These results figure out how the IRF determines a maximal depth for tomographic reconstructions and suggest criteria to select an optimal detection module for reflectance measurements.

8937-26, Session PSun

Spectrally-resolved fluorescence diffuse tomography with autofluorescence reducing technique based on symmetric measurements

Mikhail S. Kleshnin, Ilya Iosifovich Fiks, Institute of Applied Physics (Russian Federation); Ilya V. Turchin, Institute of Applied Physics (Russian Federation) and Nizhny Novgorod State Medical Academy (Russian Federation)

We present a spectrally-resolved fluorescence diffuse tomography technique which comprises: a reconstruction algorithm of fluorophore distribution in a turbid medium, a method for estimating scattering and absorption in a biotissue, and a technique for removing an autofluorescence of investigated object. The reconstruction procedure is based on the spectroscopic measurements provided by synchronous scanning of an object by a light source and a spectrometer in planar transillumination configuration. This technique utilizes the effect of fluorescence spectrum distortion while propagating through bioltissue due to the dispersion of absorption and scattering coefficients. Symmetric measurements of the investigated object, accomplished by exchanging source and detector positions can be used for removing an autofluorescence in the case of symmetric distribution of autofluorescent sources. In the other cases symmetric measurements allow to significantly reduce the autofluorescence effect. For estimating biotissue optical properties the scattering coefficient can be determined arbitrarily, but the absorption coefficient is reconstructed by using measurements of light attenuation after passing through an investigated object with the determined scattering. A hybrid model of light transfer is using to describe the light propagation in a turbid medium, and to solve the tomography inverse problem we use algebraic reconstruction technique. Numerical and model experiments have shown high accuracy reconstruction of fluorophore distribution in a turbid medium with strong autofluorescence and solution stability of the tomography inverse problem for the proposed method for estimating scattering and absorption in a turbid medium. Upcoming in vivo experiments on small animals.

8937-27, Session PSun

The use of 3D deformable optical coherence tomography and co-registered computed tomography in imaging of trapeziometacarpal articular cartilage

Paul Cernohorsky, Daniel M. de Bruin, Geert J. Streekstra, Simon D. Strackee, Ton G. van Leeuwen, Academisch Medisch Ctr. (Netherlands)

Clinical Problem:

Conventional imaging techniques are unable to depict early changes in articular cartilage associated with degenerative joint disease in small joints.

Purpose:

To depict articular cartilage of the trapeziometacarpal (TMC) joint utilizing intra-articular, 3D deformable, fiber-optic OCT and co-registered Computed Tomography (CT) in a cadaver study.

Methods:

An 18-gauge iv-cannula was inserted percutaneous into the TMC joint cavity of a cadaver wrist using standard TMC arthroscopy portals, enabling introduction of a 0.9mm thick OCT probe into the joint cavity. After positioning, helical OCT image sets were acquired using a 1300nm OCT system.

With the OCT probe in situ, a CT scan was made using a clinical wrist scanning protocol. Combined OCT- and CT scans were made for three different intra-articular probe positions.

3D deformable co-registration between OCT and CT was performed using Amira Visualization software. Imaged TMC joints were processed using a cryomicrotome imaging system, producing a high-resolution 3D model of the joint and cartilage layers for comparison.

Results:

Using OCT, Joint surfaces and cartilage-bone interfaces of both the trapezium and the first metacarpal were visualized. CT image segmentation of the trapezium and first metacarpal was performed with co-registration of three CT datasets with matching OCT datasets, producing a 3D reconstruction of the TMC joint surface. Measured cartilage thickness on OCT showed good correspondence with cryomicrotome data.

Conclusion:

Combining 3D deformable OCT and co-registered CT, successful visualization of TMC articular cartilage was performed, providing a novel approach to 3D high-resolution cartilage imaging in small joints.

8937-28, Session PSun

Dental imaging using laminar optical tomography and micro CT

Feixiao Long, Mehmet S. Ozturk, Xavier Intes, Shiva Kotha, Rensselaer Polytechnic Institute (United States)

Dental lesions located in the pulp are quite difficult to identify and, hence, to diagnose using traditional imaging methods such as dental CT. However, such lesions could lead to functional and/or molecular optical contrast. Herein, we will report on the preliminary investigation of using of Laminar optical tomography (LOT) to image the pulp and root canals in teeth. Laminar optical tomography (LOT) is a non-contact, high resolution, molecular and functional mesoscopic optical imaging modality. We demonstrate that LOT can retrieve the 3D biodistribution of molecular probes at depths up to 5mm with a resolution of several hundred microns. We injected a mixture composed of an optical dye and an x-ray contrast agent into ex vivo teeth samples and imaged them using LOT and Micro CT simultaneously. Good registration between the LOT and micro CT dye distribution was obtained, validating the potential of LOT to image non-invasively optical contrast in the teeth.

8937-29, Session PSun

Comparative sensitivity evaluation of avalanche photodiode detector and electron multiplying CCD for mesoscopic fluorescence tomography

Mehmet S. Ozturk, Xavier Intes, Rensselaer Polytechnic Institute (United States)

Optical tomographic imaging modalities for thick tissues have intrinsic limitations in sensitivity and resolution due to attenuation and scattering.

High sensitive detectors, low noise level systems are required to increase sensitivity whereas denser spatial sampling and computational methods can improve resolution. In this study, we focus on evaluating the fluorescence sensitivity of our Mesoscopic Fluorescence Molecular Tomography (MFMT) system applied to tissue engineering applications. For this purpose, we report a comparative sensitivity assessment of Avalanche Photodiode (APD) and Electron Multiplying CCD (EMCCD) for MFMT. First, we compare the sensitivity of the system using two fluorescence dyes in turbid phantoms. Second, we used live cells transfected with reporter genes to establish the minimum detectable number of cells in bio-printed tissues.

8937-30, Session PSun

High resolution 3D image reconstruction in laminar optical tomography based on compressive sensing

Fugang Yang, Shandong Institute of Business and Technology (China) and Rensselaer Polytechnic Institute (United States)

Laminar optical tomography (LOT) combines the advantages of diffuse optical tomography image reconstruction and a microscopy-based setup to allow non-contact imaging at depth up to a few millimeters. However, LOT image reconstruction paradigm is inherently an ill-posed and computationally expensive inverse problem. Herein, we cast the LOT inverse problem in the compressive sensing (CS) framework to exploit the sparsity of the fluorophore yield in the image domain and to address the ill-posedness of the LOT inverse problem. We apply this new approach to thick tissue engineering applications. We demonstrate the enhanced resolution of our method in 3-D numerical simulations of anatomically accurate microvasculature and using real data obtained from phantom experiments. Furthermore, CS is shown to be more robust against the reduction of measurements in comparison to the classic methods for such application. Potential benefits and shortcomings of the CS approach in the context of LOT are discussed.

8937-31, Session PSun

Mesh optimization in Monte Carlo-based fluorescence molecular tomography

Xavier Intes, Andrew Edmans, Rensselaer Polytechnic Institute (United States)

Fluorescence Molecular Tomography is an optical imaging technique which reconstructs the 3D distribution of a fluorescent marker in bio-tissues based on surface measurements of emitted photons and a model of light propagation. The selection of an appropriate light propagation model is critical for accurate reconstructions. Recently, computationally efficient mesh based Monte Carlo methods have been developed and employed in optical tomography applications. Herein, we investigate strategies to optimize the mesh discretization for enhanced reconstruction accuracy in small animal whole-body Fluorescence Molecular Tomography applications. We demonstrate that iterative mesh refinement in the image space allows for location and resolution improvements by applying these techniques to an anatomically correct mouse model.

8937-32, Session PSun

Structured light-based hyperspectral time-resolved diffuse optical tomography system

Qi Pian, Xavier Intes, Rensselaer Polytechnic Institute (United States)

Time-resolved Diffuse Optical Tomography (DOT) has experienced rapid progress in recent years as a powerful functional imaging technique for acquiring abundant quantitative optical information from turbid media. However, the application of time domain DOT systems is hampered by the tradeoff between gathering dense datasets and prolonged acquisition times. Recently, wide-field structured illumination patterns have been applied in time-resolved DOT platforms to significantly accelerate the data acquisition process. In this work, we present a novel structured light based imaging strategy for DOT that can generate time domain datasets enriched by hyperspectral information with short data acquisition times. We employ two digital light processors to generate wide-field imaging pattern both in the illumination and detection channels of our imaging system. The hyperspectral datasets are acquired through a multi-anode photomultiplier tube (PMT) which can detect photons in 16 wavelength channels simultaneously based on time-correlated single photon counting technique. The characteristics of the system are tested in the spatial, temporal and spectral dimensions. The performance of the imaging system is validated in vitro through the 3D absorption and scattering coefficients reconstruction of a murine model phantom. The application of digital light modulators in illumination and detection combined with time-resolved PMT spectrophotometer enables our system to acquire dense time domain datasets in an unprecedented speed. The in vitro validation shows that proposed strategy is a promising technique for fast, high resolution, quantitative three dimensional volumetric imaging.

8937-33, Session PSun

Unsupervised clustering analyzes of features extraction for a caries computer-assisted diagnosis using dental fluorescence images

Michel Bessani, Mardoqueu Martins da Costa, Univ. de São Paulo (Brazil); Emery C C C Lins, Engineering, Modeling and Applied Social Sciences Center, UFABC (Brazil); Carlos Dias Maciel, Univ. de São Paulo (Brazil)

Computer-assisted diagnosis (CAD) are performed by systems with embedded knowledge. These systems work as a second opinion to the physician, using patient data to infer diagnosis for health problems. Caries is the most common oral disease directly affecting both individuals and the society. Here we propose the use of dental fluorescence images as input data for a caries computer-assisted diagnosis using only texture descriptors; then we use statistical pattern recognition techniques to measure the descriptors performance. The data set consists of 64 fluorescence images of in vitro healthy and carious teeth including different surfaces and lesions already diagnosed by an expert. The texture feature extraction was performed on the fluorescence images using the RGB and YCbCr colors components, generating 35 different measures for each sample. Principal components analysis (PCA) was performed for data interpretation and dimensionality reduction. Finally unsupervised clustering was employed to analyze the relation between the output labeling and the diagnosis of the expert. The PCA components showed a high correlation between the features extracted; needing only six components to represent 91.9% of the original feature vectors information. The unsupervised clustering label output was compared with the classification of the expert and resulted in a match of 93.75% before and 96.88%. Beyond the significant PCA contribution for a better labeling, the unsupervised clustering results using solely texture descriptors are capable of differentiating carious and healthy teeth fluorescence signals. The use of digital image processing and fluorescence images for a caries CAD appears to be a promising approach.

8937-34, Session PSun

Comparison of LP-regularization-based reconstruction methods for early time gates in time domain fluorescence molecular tomography

Lingling Zhao, He Yang, Wenxiang Cong, Ge Wang, Xavier Intes, Rensselaer Polytechnic Institute (United States)

Time domain florescence molecular tomography (FMT) allows 3D visualization of multiple fluorophores based on lifetime contrast and provides a unique data set for enhanced quantification and spatial resolution. The time gates data set can be divided into two groups around the maximum gate, which are early gates and late gates. It is well-established that early gates allow for improved spatial resolution of reconstruction. However, photon counts are inherently very low at early gates due to the high absorption and scattering of tissue. It makes image reconstruction highly susceptible to the effects of noise and numerical errors. Moreover, the inverse problem of FMT is the ill-posed and underdetermined. These factors make reconstruction difficult for early time gates. In this work, lp (0< p≤1) regularization based reconstruction algorithmwas developed within our wide-field mesh-based Monte Carlo reconstruction strategy. The reconstructions performances were validated on a synthetic murine model simulating the fluorophores uptake in the kidneys and with experimental data obtained on a freshly euthanized mouse. We also compared the reconstructed results of different early time gate versus different signal to noise ratios of l1/16, l1/8, l1/4, l1/2 and l1 regularization methods in terms of quantification and resolution. The regularization parameters were selected by the L-curve method. The simulation results of a 3D mouse atlas and mouse experiment show that lp (0< p<1) regularization method obtained more sparse and accurate solutions than l1 regularization method for early time gates. l1/16 and l1/8 have better contrast to noise ratio than l1/4 and l1/2 .

8937-35, Session PSun

Novel fusion for hybrid optical/microcomputed tomography imaging based on natural light surface reconstruction and iterated closest point

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In mathematics, optical molecular imaging including bioluminescence tomography (BLT), fluorescence tomography (FMT) and Cerenkov luminescence tomography (CLT) are concerned with a similar inverse source problem. They all involve the reconstruction of the 3D location of a single/multiple internal luminescent/fluorescent sources based on 3D surface flux distribution. To achieve that, an accurate fusion between 2D luminescent/fluorescent images and 3D structural images that may be acquired form micro-CT, MRI or beam scanning is extremely critical. However, the absence of a universal method that can effectively convert 2D optical information into 3D makes the accurate fusion challengeable. In this study, to improve the fusion accuracy, a new fusion method for dual-modality tomography (luminescence/fluorescence and micro-CT) based on natural light surface reconstruction (NLSR) and iterated closest point (ICP) was presented. It consisted of Octree structure, exact visual hull from marching cubes and ICP. Different from conventional limited projection methods, it is 360° free-space registration, and utilizes more luminescence/fluorescence distribution information from unlimited multi-orientation 2D optical images. A mouse mimicking phantom (one XPM-2 Phantom Light Source, XENOGEN Corporation) and an in-vivo BALB/C mouse with implanted one luminescent light source were used to evaluate the performance of the new fusion method. Compared with conventional fusion methods, the average error of preset markers was improved by 0.3 and 0.2 pixels from the new method, respectively. After running the same 3D internal light source reconstruction algorithm of the BALB/C mouse, the distance error between the actual and reconstructed internal source was decreased by 0.19 mm.

8937-36, Session PSun

Diffuse fluorescence tomography based on the radiative transfer equation for small animal imaging

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Diffuse florescence tomography (DFT) as a high-sensitivity optical molecular imaging tool, can be applied to in vivo visualize interior cellular and molecular events for small-animal disease model through quantitatively recovering biodistributions of specific molecular probes. In DFT, the radiative transfer equation (RTE) and its P1 approximation, i.e. the diffuse equation (DE), have been used as the forward models. Since the DE-based DFT fails where biological tissue has a void-like region and when the source-detector separation is less than 5 mean free pathlengths, as in the situations of small animal imaging, the RTE-based DFT methodology has become a focus of investigation. Therefore, we present a RTE-based featured-data scheme for time-domain DFT, which combines the discrete solid-angle-element method and the finite element method to obtain numerical solutions of the Laplace-transformed 2D time-domain RTE, with the natural boundary condition and collimating light source model. The scheme is validated using the forward data from the Monte Carlo simulation and small-animal experiments compared to the DE-based scheme.

8937-37, Session PSun

Comparison of NIR FRET pairs for quantitative transferrin-based assay

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Transferrin (Tfn) is commonly used as a drug delivery carrier for cancer treatment. Tfn cellular internalization can be observed by Förster resonance energy transfer (FRET), which occurs when two fluorophores – donor and acceptor – are a few nanometers apart. Donor fluorescence lifetime can be used to sense and quantify FRET occurrence. In FRET state, the donor is quenched leading to a significant reduction in its lifetime. In this study, donor and acceptor near-infrared (NIR) fluorophore-labeled Tfn were used to quantify cellular internalization in breast cancer cell line (T47D). Based on donor lifetime, quantum yield and spectral data, seven NIR FRET pairs were chosen for this comparison. Performance of the different NIR FRET pairs was evaluated in vitro in multiwell plate settings and by analyzing the relationship between quenched donor fraction and acceptor:donor ratio. Additionally, we performed brightness comparison between each pairs. Several parameters, such as brightness, lifetime, R0 and FRET donor population values are used to identify the most suitable NIR FRET pair for in vivo studies in preclinical settings.

8937-38, Session PSun

Combining 3D optical and dual energy x-ray imaging to measure lipid, water, protein body composition

Serghei Malkov, John A. Shepherd, Univ. of California, San Francisco (United States)

We report on a novel technique of combining whole body 3D optical imaging and dual energy x-ray absorptiometry to estimate local body compositions in terms of lipid, water and protein masses. To validate our methods we designed phantoms with tissue-like properties as our reference standards. The calibration was created by fitting phantom values using non-linear regression of quadratic and truncated cubic polynomials. Dual-energy measurements were performed using a commercial bone densitometer system. The phantoms were made of materials shown to have similar x-ray attenuation properties of the biological compositional compartments. The three-dimensional body shape was reconstructed from the depth maps generated by Microsoft Kinect for Windows. We used open-source Point Cloud Library and freeware software to produce dense point clouds. Accuracy and precision of compositional and thickness measures were calculated. We are able to achieve accuracy within 2% for lipid and water and about 5% for protein. The error contributions due to two modalities were estimated. The preliminary phantom composition and shape measurements are found to demonstrate the feasibility of the method proposed.

8937-39, Session PSun

Quantitative determination of the signal and resolution limits of Cerenkov luminescence imaging

Justin S. Klein, Gregory S. Mitchell, Simon R. Cherry, Univ. of California, Davis (United States)

Many medically-relevant beta-emitting radionuclides produce small numbers of optical photons via Cerenkov luminescence (CL), which are emitted along the particle's trajectory following radioactive decay. Recent interest in this phenomenon has led to in-vivo Cerenkov luminescence imaging studies, yet many of the efforts have been qualitative and proof-of-concept. CL holds immense promise as a high-throughput, low-cost means for optical imaging of radiotracers. However, a thorough, quantitative treatment of Cerenkov luminescence, is needed to provide insight into the possible applications and limitations of this new imaging modality.

We used Monte Carlo to model the generation and transport of CL emitted from C-11, F-18, I-131, Rb-82 and Y-90 and determined the depth-dependence of signal (luminescence energy reaching the surface per decay) and spatial resolution in various tissues. There was an inverse relationship between signal and resolution. For example, at a depth of 1 mm in simulated brain tissue, I-131 had the lowest signal (0.37 eV/decay) and the best resolution (1.2 mm). Conversely Rb-82, under the same conditions, produced a signal of 65.50 eV/decay but had the poorest resolution of 2.4 mm. We also made comparisons against direct beta particle detection. The spatial resolution for imaging beta particles was better than CL at all depths simulated. The signal from direct beta detection decreased rapidly with depth. Beta particles emitted from F-18 yielded an average signal of 2260 eV/decay at 1 mm decreasing to 0.97 eV/decay at 2 mm depth. At depths >~3 mm the CL signal exceeded that from direct beta particle detection.

8937-40, Session PSun

Developing and testing a multisource and detector reflectance diffuse optical tomography system

Murat Canpolat, Hüseyin Özgür Kazanci, Tanju Mercan, Akdeniz Üniv. (Turkey)

A continues wave reflectance diffuse optical tomography (rDOT) system with an optical fiber probe, including 49 sources and 49 detectors has been developed and tested using breast tissue phantoms with inclusions. All the source and detector fibers are located on a 10 ? 10 grids with dimensions of 28 mm ? 28 mm, and the source and detector fibers are separated by three mm. In total, there are 22 different source-detector distances on the probe. The system has a high dynamic range due to the six different integration times for each detector channel. A calibration method has been developed to calibrate multi source and detector fiber probe. Data acquired from a homogenous tissue phantom was a mixture of 1% intralipid and indocyanine green (ICG) with a reduced scattering (?s) and an absorption (?a) coefficients of 1 mm?1 and 0.02 mm?1, respectively. The inclusions were prepared using the intralipid and ICG mixture to have scattering and absorption coefficients of 1 mm?1 and 0.073 mm?1, respectively. The tip of the probe was placed on the surface of the tissue phantom, and the inclusion was placed at depths of 5, 10, 15, and 20 mm in the intralipid mixture then the data were acquired. For the reconstruction, the perturbation data were obtained in two different ways. The first was by direct subtraction: the measurements of the tissue phantom acquired without the inclusion were subtracted from those with the inclusion. In the second method, an average over the measurements with the same s-d separation was calculated, and this was then subtracted from the original measurements and repeated for all s-d separations. The tissue phantoms were reconstructed using Tikhonov regularization. The depth compensation algorithm (DCA) was used to increase the accuracy of the localization of the inclusions at different depths.

8937-41, Session PSun

On the use of Cramér-Rao bounds for diffuse optical imaging system design

Vivian E. Pera, Dana H. Brooks, Mark J. Niedre, Northeastern Univ. (United States)

A persistent topic in diffuse optical imaging is how best to design source and detector configurations. Singular value analysis is conceptually straightforward, but it does not make use of the information contained in the singular vectors of the Jacobian (sensitivity matrix) or the covariance of the measurements. An alternative is the Cramér-Rao lower bound (CRLB), which defines the best achievable precision of any estimator and has long been used in the statistical signal processing community to optimize system design. Computing the CRLB requires inverting the Fisher information matrix (FIM), however, which is ill-conditioned in the case of diffuse optical tomography. We previously examined a common way of regularizing the FIM (i.e., the point-target assumption) and found the resulting CRLBs not to be useful predictors of performance across different system configurations.

Here we report on additional analyses that confirm and extend our previous results to other image reconstruction methods and to different ways of regularizing the FIM. We evaluated the performance of a two-dimensional circular imaging geometry for various detector apertures and a Poisson noise model using the randomized algebraic reconstruction technique (r-ART) and found that as before, the point-target CRLBs were unable to predict performance trends across different source-detector configurations. We also considered the applicability of the Bayesian CRLB and concluded that the degree of regularization necessary to obtain reasonable solutions to the inverse problem limited the utility of this approach. Finally, we examined the impact of different methods of computing the Jacobian on our results.

8937-42, Session PSun

Simulation of optical breast density measurements using structured light illumination

Jessica Kwong, Farouk Nouizi, Yifan Li, Min-Ying Su, Gultekin Gulsen, Univ. of California, Irvine (United States)

Breast density is a risk factor for breast cancer and we propose using Diffuse Optical Imaging with structured light illuminations (SLI) to quantify the percentage of the fibroglandular (dense) tissue within the breast. Segmentations of dense tissue from four MRI cases were used to create a geometric model of the breast and its dense tissue. A Comsol-generated Finite Element (FEM) mesh was used for simulating diffusion-based light propagation through the breast tissue and reconstructing the absorption maps [1]. In these preliminary simulations, the absorption coefficients of the non-dense and dense tissue were assigned using literature values and synthetic SLI measurements were obtained using the FEM-based forward solver. During the simulation, 12 distinct illumination patterns were generated and consisted of vertical stripes, horizontal stripes, and a checkerboard pattern. Integration of the same patterns on the detection site allowed for single point measurements, increasing the number of measurements, the combination of illumination pairs, to 144 (12 x12). Using these simulated measurements, FEM-based inverse solvers were used to reconstruct the 3D absorption maps. The number of dense tissue (high absorption) elements within the reconstruction was calculated and used to compute the percentage of dense tissue in the breast. These densities correlated with those determined from the MRI segmentation analysis (r = 0.9866). We are currently continuing these simulations with additional cases and expanding the simulation and reconstruction algorithms to determine the concentrations of multiple chromophores (water, lipids, oxy- and deoxy-hemoglobin) within the dense and non-dense breast tissue.

Conference 8938: Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XIV

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8938 Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XIV

8938-1, Session Key

Fiber optics sensing *(Keynote Presentation)*

Wojtek J. Bock, Univ. du Québec en Outaouais (Canada)

No Abstract Available

8938-56, Session Key

MINERVA European Consortium: MId- to NEaR infrared spectroscopy for improVed medical diAgnostics: aspirations and progress *(Keynote Presentation)*

Angela B. Seddon, The Univ. of Nottingham (United Kingdom)

The European Consortium: MINERVA, standing for MId- to NEaR infrared spectroscopy for improVed medical diAgnostics, has a € 7M ($9.5M) portfolio and runs 2012 to 2016. This is a Large-scale integrating project (IP) addressing the Work programme topics: Objective ICT-2011.3.5 Core and disruptive photonic technologies: a) Core photonic technologies: 2. Biophotonics for early, fast and reliable medical diagnosis. The 12 Project Partners are a mix of academics, medical scientists and industrialists. The University of Nottingham, UK, is making the mid-infrared (mid-IR) fibreoptics for this program. Two specific high impact applications will be addressed: high volume pathology screening (i.e. automated microscope-based examination of samples) and in vivo, remote, real-time skin surface examination (i.e. non-invasive investigation of suspected skin cancer). This project will be instrumental in opening the mid-IR to further exploitation, and the technology developed will be transferable to a huge range of applications both in bio-photonics and in wider industry. Here, Project aspirations will be described, along with progress.

8938-2, Session 1

Needle-tip localization using an optical fiber hydrophone

Jean Martial Mari, Univ. College London (United Kingdom); Simeon West, Univ. College Hospital (United Kingdom); Benjamin T. Cox, Paul C. Beard, Adrien E. Desjardins, Univ. College London (United Kingdom)

Accurately and efficiently guiding a medical device such as a needle or a catheter to a target in the human body is of critical importance in a wide range of minimally invasive procedures, such as peripheral nerve blocks, central venous catheterizations, biopsies, and electrode insertions. B-mode ultrasound is commonly used for guidance, but visualization of the device tip can be chal-lenging. Needle and catheter tips readily deviate from the imaging plane and they can have poor echogenicity at steep insertion angles. Complications arising from loss of device tip visibility can be life-threatening if a critical structure is punctured or if anesthetics are injected directly into the blood stream. In this study, we present a new method for identifying and tracking medical devic-es within the imaging plane that involves the optical detection of ultrasound. With this method, ultrasound pulses for localization are transmitted by the imaging probe and received by a fiber-optic hydrophone that is integrated within the medical device. Custom software was developed to generate ultrasound pulses for device localization, to receive signals from the hydrophone, and to acquire ultrasound images. With the present system, localization accuracy is 0.39 ± 0.28 mm in the lateral dimension and 0.25 ± 0.11 mm in depth. While this level of accuracy is greater than what is required for clinical practice, the potential for improvements based on the application of curve fitting algorithms to cross-correlated hydrophone signals is discussed. The method pre-sented in this study has strong potential to increase procedural efficiency and safety in a manner that is compatible with current clinical workflow.

8938-3, Session 1

Selenide and telluride glasses for mid-infrared bio-sensing *(Invited Paper)*

Shuo Cui, Radwan Chahal, Yaroslav Shpotyuk, Université de Rennes I (France); Catherine Boussard-Plédel, Jacques Lucas, Univ. de Rennes 1 (France); Frederic Charpentier, Hugues Tariel, DIAFIR (France); Olivier Loréal, Université de Rennes I (France); Virginie Nazabal, Univ. de Rennes 1 (France); Olivier Sire, Université de Bretagne Sud (France); Valérie Monbet, Université de Rennes I (France); Zhiyong Yang, Pierre Lucas, University of Arizona (United States); Bruno Bureau, Univ. de Rennes 1 (France)

Chalcogenide glasses exhibit large transmission in the mid-infrared region. These glasses could be easily shaped into optical devices such as lenses and optical fibers. During the past decade, selenide glass fibers have been proved to be suitable for infrared sensing in an original spectroscopic method named Fiber Evanescent Wave Spectroscopy (FEWS). Once coupled to unsupervised statistical analysis techniques, FEWS has provided very nice and promising results, especially for biology and medical diagnosis. Compared to other optical fibers, selenide glass fiber are efficient and sensitive due to, first, the shaping of original probing head, and, second, to the hydrophobic behavior of the fiber thanks to the covalent nature of the chemical bonds forming the glass. Moreover, these fibers have been shown to be bio-compatible by passing bio-toxicity official tests. Thanks to these results, the DIAFIR Company has been founded (http://www.diafir.com/) which develops optimized fibered devices increasing the sensitivity of the protocol.

In parallel, some rare earth doped chalcogenide glass fibers have been developed. For example, sulfide glass doped with Dy3+ emits a broad band ranging from 4 to 5 µm. They were used as secondary remote sources for infrared sensing.

Also, new telluride glasses have been discovered opening the transmission toward 20 µm and suitable for molecule sensing absorbing beyond 12 µm.

At last, chalcogenide microstructured optical fibers combining plain core and ring of holes around the core were developed.

In the next future, it is planned to use these new sophisticated fibers in the frame of medical applications.

8938-4, Session 1

Effects of sterilization on optical and mechanical reliability of specialty optical fibers and terminations

Andrei A. Stolov, Edward T. Warych, William P. Smith, Paula L. Fournier, Adam S. Hokansson, Jie Li, R. Steve Allen, OFS (United States)

Optical fibers and terminations were subjected to different sterilization techniques, including multiple autoclaving and treatments with peracetic acid, e-beam and UV radiation. Effects of different sterilization techniques on key optical and mechanical properties of the fibers and the terminations were revealed. The primary attention was given to behavior of the coatings on the fibers and adhesives used in the terminations in harsh sterilization environments. The optical fibers with following four coating/buffer types were investigated: (i) dual acrylate, (ii) polyimide, (iii) silicone/PEEK and (iv) fluoroacrylate hard cladding/ETFE.

8938-5, Session 1

Fiber optic enables point of care bioanalysis 250 miles from earth

Ozzy Mermut, Christophe Riviere, Jessie Weber, Mathieu Legros, Paul Grenier, Pierre Chartrand, Pascal Gallant, INO (Canada); Geneviève Dubeau-Laramée, Luchino Y. Cohen, Isabelle Jean, Derrick Piontek, Daniel Provençal, Canadian Space Agency (Canada)

Clinical assessment of cells and biomarkers can readily be performed with the versatile and widespread technique of flow cytometry. Typically constrained to sophisticated laboratories, translation of flow cytometry instrumentation from the bench to the field, where access to centralized care and testing infrastructures are limited, make remote implementations of such complex biophotonics systems nontrivial. Unusual environments and operational contexts, such as manned space missions and exploration, further bring new challenges in designing and using remotely deployable bioanalytical sensors. In such settings, prolonged weightlessness triggers physiological and immunological adaptation in astronauts. An autonomous and simple to use flow cytometer would be a vital tool for monitoring stress and immune biomarkers in space where diagnostic and monitoring technologies are limited, and where visits to physicians are not an option. To this aim, we present Microflow, a sheathless fiber optic flow cytometer based on a unique optofluidic fiber flow cell platform. Featuring near real-time and automated push-button-and-go operation, the Microflow sensor is ideal for point-of-care medicine and molecular biology research 250 miles above earth. This fiber optic innovation demonstrates excellent performance characteristics employing calibrated microfluorospheres, when tested on the ground and during parabolic flight campaigns. The first ever biomedical technology demonstration of a fiber optic flow cytometer by an astronaut in the International Space Station (ISS) in March 2013, is presented. Development of this miniature portable fiber optics platform from concept to implementation as well as results from first measurements of immunological and biomedical markers (CD45, CD4, Th1/Th2 cytokines) from aboard the ISS is discussed.

8938-6, Session 2

Fabrication of 50-µm-bore hollow fiber for infrared transmission

Katsumasa Iwai, Kouki Takahashi, Hiroyuki Takaku, Sendai National College of Technology (Japan); Mitsunobu Miyagi, Tohoku Institute of Technology (Japan); Yi-Wei Shi, Fudan Univ. (China); Yuji Matsuura, Tohoku Univ. (Japan)

Extremely flexible hollow fibers with 50 ?m-bore size were developed for infrared laser delivery. The hollow fiber was inner coated with silver and a dielectric layer to enhance the reflection rate at an objective wavelength band. The silver layer was inner-plated by using the conventional silver mirror-plating technique. Concerning the fabrication parameters used up to now for 320-?m bore-sized fibers, the target flowing rate for plating solutions was 10 ml/min. Parallelly arranged bundles of silica capillary were used to increase the cross-sectional area. To achieve the target, bundles with 4800 pieces were used for the capillary with a length of 20 cm and inner diameters of 50-?m. The loss for the 50-?m bore size, 3-cm length silver hollow fiber was 6 dB at the wavelength of 1 ?m. Thin dielectric layer was formed by using liquid-phase coating method for low-loss transmission of Nd:YAG and Er:YAG laser light.

8938-8, Session 2

Monitoring Biofilm Attachment on Medical Device Surfaces Using Hyperspectral Imaging.

Hanh ND Le, Victoria M Hitchins, Wenhui Zhang, Ilko Ilev, Do-Hyun Kim, US Food and Drug Administration (United States)

Through a protective extracellular matrix containing polysaccharides, protein and DNA, a bacterial community can not only form strongly adhered stacks but also expand to neighboring surfaces. In addition, the coating enables biofilms to persist on implants such as prosthetic heart valves, peritoneal dialysis catheters, or implanted joint prostheses. To monitor biofilm attachment on different surfaces, hyperspectral imaging was utilized for distinguishing the spectral differences. Examination of coupons made of several different medical device materials revealed the presence of biofilms. Furthermore, the examination indicated the ability of various device materials to affect the biofilm production. In this study, hyperspectral imaging was used to investigate the effects of shear stress on the adhesion of biofilms on common medical device surfaces such as polytetrafluoroethylene, stainless steel, titanium, polycarbonate and glass. Following the growth of Pseudomonas aeruginosa after 24, 48 and 72 hours at 37o C, the surfaces containing biofilms were tilted at 10, 45 and 90 degree for 30 seconds to induce shear stress and were examined employing a hyperspectral imaging approach. Our hypothesis was that stronger attachment would be able to withstand greater shear stress. Experimental data demonstrating this hypothesis will be presented.

8938-9, Session 2

Improved deep UV optical fiber for medical and spectroscopy applications

John H. Shannon, Valery Khalilov, Richard J. Timmerman, Polymicro Technologies (United States)

An effort to reduce UV-induced defect centers and improve the UV solarization resistance in a high –OH synthetic fused silica step index multimode optical fiber, designated as FDP, was successfully completed at Polymicro Technologies. The development achieved significant reduction in the 214 and 265nm absorption bands typically associated with solarization affects in fused silica. The improvements were applied to fiber sizes from 68 to 600µm. Characterization of the solarization resistance was performed with added attenuation from UV exposure demonstrated to be less than 1dB per two meters tested for all fibers in the core size range. Results of spectral performance and UV degradation are presented along with a description of the test protocols. Potential applications in the medical and spectroscopy fields also will be discussed.

8938-55, Session 2

Design of graded index fiber for fiber-optics probe in OCT applications

Xiaoguang Sun, Jie Li, OFS (United States)

A typical fiber optic probe for optical coherence tomography (OCT) applications is made of a short piece of graded index (GRIN) optical fiber fused onto a single-mode fiber. The GRIN fiber is used to focus the output light from the SM fiber onto a sample as a lens. Simple

modeling of such a probe can be done using a Gaussian beam ABCD method to predict the optical properties of the probe. However, when the assumptions of Gaussian power distribution for the beam from a single mode fiber and parabolic index profile for the GRIN fiber are considered invalid, we need to use a beam propagation method (BPM), which does not require these assumptions, to simulate the optical characteristics of the probe. In this paper, we have used the BPM approach and analyzed some important properties of the probe such as lateral resolution, working distance and coupling efficiency of the reflected light. We have then compared the calculated properties with the measured ones and achieved reasonable agreement between the two.

8938-10, Session 3

Effect of diffusivity in calculating photothermal damage of tissue embedded with small heat-absorbing particles

Hanh ND Le, Do-Hyun Kim, US Food and Drug Administration (United States)

Evaluation of optical radiation hazard of medical optical devices is one of the most important aspects in assuring their safe operation. Previously, we adapted an analytical solution of heat diffusion equation to calculate different photothermal damage thresholds from pulsed and scanning light sources. In this work, we expand our analytical computational model to address different effects of diffusivity in calculating photothermal damage threshold of tissue. The tissue is embedded with small heat-absorbing particles, such as melanosome and gold nano-particles. Melanosomes are considered as small particles compared to gold nano-particles, which can be approximated as point heat absorbers. The effects of diffusivity are numerically simulated and indicate that the heat contained and sustained in the tissue volume exhibit noticeable change of thermal damage threshold of different types of tissue. Further discussion of applying this study in evaluation of gold nano-particle assisted optical therapeutic devices will be provided.

8938-11, Session 3

Dual optical coherence tomography and infrared thermography imaging system

Israel Gannot, Tel Aviv Univ. (Israel)

Multimodal imaging systems incorporate several imaging modalities combined in order to simultaneously image a tissue. These systems are a promising platform for clinical imaging, allowing imaging of the tissue's physical structure as well as its functionality.

Different pathologies such as vulnerable plaques can induce functional and structural changes. Functional changes such as the formation of internal endogenous heat sources (inflammatory regions) can affect the surface temperature. Structural changes in a tissue generate refractive index mismatch and induce light backscattering from layers at different depths.

This work presents a bimodal imaging system consisting of an optical coherence tomography (OCT) and thermography modalities. An experimental setup was built to test tissue layered phantoms, with and without a variable-power, subsurface heat source. A thermal camera was used to measure surface temperature variability and detect internal heat sources. A swept source full field OCT was implemented using a CW Ti:Sapph Tunable laser, in order to image tissue's structural changes. Setup mechanical motion was avoided due to the use of wide field illumination and wavelength scanning. Multiple A-scans were taken simultaneously using a CCD camera. System's depth resolution and scan time are dynamic, changeable by varying the wavelength scan range.

8938-12, Session 3

New glass developments for fiber optics

Paige L. Higby, SCHOTT North America, Inc. (United States); Karen Holst, SCHOTT AG (Germany); Kevin Tabor, William James, Elizabeth Chase, Sally Pucilowski, Elizabeth Gober-Mangan, Ronald Klimek, SCHOTT North America, Inc. (United States); Frank Karetta, Bianca Schreder, Schott AG (Germany)

Fiber Optic components for Lighting and Imaging applications have been in use for decades. Recent requirements such as a need for RoHS compliance, lower costs, or particular optical properties such as NA or Transmission have required SCHOTT to develop and implement new glasses for these applications. From Puravis™ lead-free fibers for lighting applications, to new glasses for digital X-ray imaging and sensor applications, the challenges for SCHOTT scientists are considerable. Pertinent properties of these glasses, and methods of determination of suitability will be discussed.

8938-13, Session 3

Grazing angle sensing approach in fiber-optic Fourier transform infrared (FO-FTIR) spectroscopy for detecting surface contamination

Moinuddin Hassan, Ilko Ilev, U.S. Food & Drug Administration (United States)

Medical device contamination has become a critical and prevalent public health issue as the devices are being extensively used in clinical practices for diagnostics, therapeutics and as medical implants. In order to prevent transmission of infection, development and implementation of novel test methods for quantitative, accurate, easy-to-use and real-time detection of contaminations is a critical requirement. Conventional clinical methods are time consuming and complex. Recently, we have demonstrated a novel proof-of-concept platform for label-free, remote and rapid detection of medical device surface contamination based on a fiber-optic Fourier transform infrared (FO-FTIR) spectroscopy method. In this study, employing the FO-FTIR platform, we present a grazing-incidence angle (GIA) sensing probe approach for remote and in-situ detection of biochemical contaminant on medical device surfaces with enhanced sensitivity and specificity in the mid-infrared (MIR) spectral range of 2.5 ?m to 11 ?m. The GIA sensor head consists of two MIR flexible hollow-core fiber arms including a delivery fiber and a signal fiber to change incident angle (grazing angle) from 70o to 85o. The sensitivity of the MIR reflected measurements is maximized for thin layers of biochemical contaminants on any type of metallic and dielectric material surfaces. The test measurements were performed in a GIA reflection mode with different types of contaminants on different types of surfaces. Standard and protein samples of different concentrations under dry conditions were also used to investigate the sensitivity of the system. The results were validated by comparing with alternative spectroscopic measurement modes such as reflection and transmission.

8938-14, Session 3

Improvement of specialty fiber damage at 266 nm wavelength

Karl-Friedrich Klein, Tim Tobisch, Hannah Ohlmeyer, Technische Hochschule Mittelhessen (Germany); Hartmut Zimmermann, CryLas GmbH (Germany); Georg Hillrichs, Hochschule Merseburg (Germany)

Multimode UV-fibers with core diameters from 70 to 600 µm diameter

have been mainly characterized and, in parallel, improved due to fiber drawing and processing using deuterium lamp with a broadband spectrum. In meantime, UV-induced losses in these fibers have been reduced to levels below 0.8 dB, for 2 m long samples.

On the other hand, damaging studies with high-power excimer-lasers at 193, 248 and 308 nm wavelength have been carried out. For attractive applications, e.g. in analytics or sensing, compact and less expensive laser-systems and LED-systems are available. As already shown, even pulsed 355 nm Nd-YAG lasers generate UV-damage below 280 nm: especially the E'-centers at 214 nm and the NBOHC at 260 nm are generated.

So far, there is no multi-mode or low-mode demonstrated with high UV resistance at the wavelength of operation (266 nm). In this report, a double-clad fiber with 70 µm core diameter will be introduced. The laser-induced UV-damage will be compared between 70 µm core fiber and other improved UV fibers with similar core sizes. In addition, the above results will compared with those using broadband cw lamps.

8938-15, Session 3

Integrated optical fiber shape senor modules based on twisted multicore fiber grating arrays

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Shape sensing using optical fibers has the potential to impact various medical, industrial and defense related applications. Optical fiber shape sensing is a form of distributed sensing that uses scattered signals from a multi-waveguide structure, e.g., a multicore optical fiber, to ascertain local curvature and thus the shape of the length of fiber under interrogation. A key component of this system is a compact, integrated optical fiber assembly that allows sufficient sensitivity, collection efficiency and low cross-talk of the scattered signals.

In this paper we report on the development of a complete integrated optical fiber assembly suitable for shape sensing. Our shape sensor module consists of a length (>1m) of twisted multicore optical fiber with discrete or quasi continuous fiber Bragg gratings inscribed along its length. Our fiber has a compact 180 micron diameter, a twist of 50 turns per meter, and grating reflectivities greater than 0.01% per cm of array, suitable for high efficiency scatter measurements over many meters of fiber. A tapered fiber bundle single core to multicore fanout can be either spliced or connectorized to this shape sensor array. Termination of the fiber grating array ensures low back reflection from the distal endface of the fiber array and thus minimal interference with sensor interrogation.

Our shape sensor modules can be combined with many different interrogator schemes and represent a complete optical sensor head solution for medical, industrial and defense applications.

8938-16, Session 4

Silver-coated Teflon hollow waveguides for the delivery of terahertz radiation

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Significant research exists regarding the successful implementation of hollow waveguides for the low-loss transmission of infrared radiation in applications ranging from laser power delivery to spectroscopy. With the continued development of terahertz (THz) technologies and applications, it is often advantageous to have a waveguide analogous to the IR hollow waveguides for the transmission of THz radiation. This study focuses on the fabrication of novel silver-coated polytetrafluoroethylene (PTFE) waveguides for the transmission of terahertz radiation. The hollow structure described in this paper is made by depositing a thin film of Ag on the outer surface of a dielectric (Teflon) tube. This is in contrast to depositing metallic and dielectric thin film coatings on the inner surface of capillary tubing as is commonly done for IR and some THz transmissive waveguides. In this work, the Teflon tubing itself is the dielectric layer that is used to enhance the reflectivity of the Ag. Theoretical loss calculations will be presented and compared to the loss obtained for the guides measured at THz frequencies. In addition the spectra of the guides in the infrared region are also measured as a means to study the uniformity of the Teflon "layer" and to confirm the wall thickness of the Teflon tubing. The surface topography of the silver/PTFE waveguides is obtained and the resulting surface roughness related scattering losses are calculated. The implications of the terahertz fiber for applications ranging from nondestructive evaluation (NDE), security, and medical imaging are briefly discussed.

8938-17, Session 4

Multi-channel near-infrared spectrometer for functional depth-resolved tissue examination and positioning applications

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Interferometric depth-sensing techniques using broadband laser sources such as optical coherence tomography or low coherence interferometry are well established for morphological imaging of biological tissue and find successively more technical applications. Several techniques, to provide enhanced or functional information have been investigated before. Polarization sensitive measurements help to highlight anisotropic or depolarizing regions; noise and speckle reduction methods and techniques to eliminate the mirror artifacts arising from non-complex-valued detection have been proposed and individually demonstrated.

All these image methods have in common that they require parallel and preferably simultaneous acquisition of multiple signals with a long-term stable device. To simplify and improve existing high speed instruments we present a broadband near-infrared spectrometer offering up to eight parallel acquisition channels utilizing a V-Groove fiber mount for 8-channel input and a fast 2D CCD camera capable of dynamically selecting and optimizing the different channels. Preliminary characterizations with a polarization sensitive detector indicate a good match between simulated and actual performance of the device with a central wavelength of 840 nm. We achieve a depth resolution of 5.4 µm in air with a signal roll-off of 19.9 dB over a depth range of ±3.21 mm. The stable phase correlation between individual channels is provided by the compact and rigid parallelized set-up. It can be used as an attractive development platform for integrating different acquisition schemes, but also acts as compact high-speed, multi-sensor ranging device with nanometer scale accuracy for biomedical and engineering applications.

8938-18, Session 4

Multi component extruded crystalline fibers for 3-15 µm

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New multi materials low loss extruded micro- and nano structured metal halides crystalline fibers for 3-15 μ m IR region will be presented for the fist time. Alkali metal halides core and multi cladding composite fibers were extruded with losses from 0.5 dB/m to 0.7 dB/m at 10.6 μm. Fibers are flexible, temperature and time stable up to 30 W cw power delivery of CO2 laser, water protected and may use as fiber scalpel under intense UV illumination. Low loss multi-mode core with multi cladding and with metal, semiconductor and nano diamond impregnation will be presented for the fist time.

8938-19, Session 4

Microstructured optical fiber-based micro-cavity sensor for chemical detection

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The studies on microstructured optical fibers (MOF) have drawn considerable interest and played an important role in many applications. MOFs provide unique optical properties and controllable modal properties because of their flexibilities on manipulation of the transmission spectrum and the waveguide dispersion properties. MOFs are especially useful for optical sensing applications because the micro-structured air channels in MOF can host various types of analytes such as liquids, gases, and chemical molecules. Recently, many studies have focused on the development of MOF-based optical sensors for various gases and chemical molecules. We propose a compact, and highly sensitive optical micro cavity chemical sensor using microstructured fiber. The sensor probe is composed of a hollow optical fiber and end cleaved microstructured fiber with a suspended core. The interference spectrum resulting from the reflected light at the pure silica and air interfaces changes when the micro cavity is infiltrated with external chemical molecules. This structure enables the direct detection of chemical molecules such as volatile organic compounds (VOCs) without the introduction of any permeable material. Chemical vapor sensing experiments for the various analytes delivered in both static flow mode and dynamic flow mode will be demonstrated. The sensitivity, the stability, and the potential biomedical and industrial applications of the proposed scheme will be discussed.

8938-20, Session 4

5-mm piezo-actuated fiber endoscope for high-speed ultrafast laser microsurgery

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We developed a 5-mm diameter endoscope for high speed ultrafast laser microsurgery. The endoscope consists of an air-core photonic bandgap fiber (PBF) for the delivery of high energy pulses, a piezoelectric tube actuator for fiber scanning, and two aspherical lenses for focusing the light. Its inline optical architecture provides easy alignment and substantial size reduction to 5-mm diameter as compared to our previous MEMS-scanning probes while realizing improved lateral and axial resolutions of 1.16 ?m and 11.35 ?m, respectively. We studied the maximum pulse energies that could be delivered through a 1-m PBF and identified cladding damage at the input facet as the limiting factor. The maximum pulse energy that can be coupled depends on the coupling NA, beam profile, and misalignments of the beam during coupling. Using different NA`s and considering radial misalignment of the beam, we found the damage threshold of the cladding to be an order of magnitude lower than that of the bulk silica. This lower damage threshold might possibly be attributed to the near field enhancement of the coupled beam at the sharp edges of the nanostructured holey region. The use of a 300 kHz repetition rate fiber laser enabled rapid ablation of 150 μm x 150 μm area within only 50 ms, corresponding to a 0.25 cm2/min tissue removal speed. Using this probe, we will present laser drilling through scarred cheek pouch tissue. With further development, this probe can serve as a precise and high speed ultrafast laser scalpel in the clinic.

8938-21, Session 4

Low-temperature, low-cost growth of robust ATR GeO2 hollow fibers based on copper capillary tubes for transmission of CO2 laser light

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Attenuated total reflection (ATR) infrared hollow waveguide attracts particular interest since it has both advantages of a hollow fiber and a light guiding mechanism similar to that of solid-core fibers. Presently, ATR hollow waveguides are mainly structured with single-crystal sapphire or glassy materials. These waveguides are somewhat brittle. More robust ATR hollow fibers are required in many military and domestic applications. In this work, ATR GeO2 hollow waveguides were prepared based on a copper capillary tube for transmitting CO2 laser light. The inner wall of the copper structural tube was polished to a mirror-smooth state by using high-pressure pulsed nanofluid technology. A crystallized GeO2 reflective film with sufficient thickness (>4 ?m) was grown on the inner wall of the copper tube via a homogeneous liquid phase deposition process at room temperature. 2-meter-long ATR GeO2 hollow fibers were obtained. This type of hollow waveguide has high mechanical strength and heat resistance. It can still be bent since the hollow-core size (1.4 mm) and the wall thickness (50 ?m) are not too large. The transmissions of CO2 laser light are 91% and 43% under a straight condition and a 90° bend with a 30-cm radius condition, respectively. The waveguide has a high laser damage threshold due to good thermal conductivity of the copper substrate tube and a high melting point (1115oC) of the GeO2 reflective layer. This work opens a door for low-temperature, low-cost growth of long ATR GeO2 infrared hollow fibers based on various substrate tubes, even including plastic capillary tubes.

8938-22, Session 4

Towards simultaneous and co-localized optical frequency domain imaging and laser therapy through a double clad fiber

Kathy Beaudette, Ecole Polytechnique de Montréal (Canada) and Massachusetts General Hospital (United States); Hyoung Won Baac, Harvard Medical School (United States) and Massachusetts General Hospital (United States); Wendy-Julie Madore, Etienne De Montigny, Ecole Polytechnique de Montréal (Canada); Martin L. Villiger, Harvard Medical School (United States) and Massachusetts General Hospital (United States); Nicolas Godbout, Ecole Polytechnique de Montréal (Canada); Brett E. Bouma, Harvard Medical School (United States) and Massachusetts General Hospital (United States); Caroline Boudoux, Ecole Polytechnique de Montréal (Canada)

The use of double clad fibers (DCF) and double clad fiber couplers (DCFC) could enable simultaneous optical frequency domain imaging (OFDI) and laser therapy in a co-localized manner. The DCF allows a single channel fiber-optic probe to be shared: i.e. the core delivers the OFDI signal while the clad guides the therapy light. Here, we investigated possible degradation mechanisms of the OFDI signal through the DCF and the DCFC. Then, we evaluated the coupling performance of a therapy light with a preliminary setup. The therapy light is provided by a high-power Raman fiber laser that is designed to operate at 1436-nm wavelength (CW-modulated; <0.5 W average power). A DCFC was used to combine the therapy light with the OFDI signal. The DCFC allows achromatic transmission (> 95%) of the core light in the OFDI band (1265-1325 nm) as well as the coupling (~38%) of the inner cladding light. The DCFC can support an average power of up to 1 W. The Raman fiber was directly spliced to the DCF with a slight lateral misalignment (~10 μm) so that the core of the Raman fiber faced the inner cladding of the DCF. Due to the symmetric coupling ratio in the two arms, in the current DCFC configuration, about 35% of the laser input was delivered through the inner cladding to the output port. A multi-modal system allowing simultaneous and co-localized ODFI and laser therapy would enable real-time monitoring of laser thermal or ablation treatment of epithelial lesions in pathologies such as Barrett's esophagus.

8938-7, Session 5

Gold nanoparticles sensing with diffusion reflection measurement as a new medical diagnostics application *(Invited Paper)*

Dror Fixler, Bar-Ilan Univ. (Israel)

The ability to quantitatively and noninvasively detect nanoparticles in vivo has important implications on their development as optical sensors for medical diagnostics. We suggest a new method for cancer detection based on diffusion reflection (DR) measurements of gold nanorods (GNR). The DR method is a simple, noninvasive imaging technique which has been proven useful for the investigation of the optical parameters of the tissue. In our talk, the ability to extract optical properties of phantoms and their GNR concentrations from DR measurements will demonstrate. We will present the detection of tumor in depth of 7 mm in the tissue. We will report, for the first time, GNR detection through upper tissue-like phantom layers, as well as the detection of a tumor presented as highly concentrated GNR placed deep within a phantom.

8938-23, Session 5

Photothermal tissue coagulation using pulsed Raman fiber laser at 1.44-μm wavelength

Hyoung Won Baac, William Lo, Martin L. Villiger, Brett E. Bouma, Harvard Medical School (United States)

In vivo diagnostic imaging has been available in various forms, utilizing optical, ultrasonic, and photoacoustic modalities. However, many clinical applications require longitudinal surveillance of suspicious lesions after detection by these imaging techniques. For this purpose, spatio-temporal localization of such suspicious lesions by means of visible or traceable marks would be highly desirable. Photothermal tissue coagulation offers an efficient strategy to generate highly reproducible marks on suspicious lesions with fine precision, and avoid potential issues of ablation-based approaches such as catheter compatibility or tissue relocalization.

We have developed a high-energy pulsed fiber Raman laser operating at 1.44-?m wavelength (>20 mJ/pulse). Currently, we intend to use this marking laser in combination with optical frequency domain imaging (OFDI) for image-guided biopsy in Barrett's esophagus. The wavelength was specifically designed to match the optical absorption peak of water for efficient tissue coagulation. In this presentation, we demonstrate tissue coagulation ex vivo under various exposure conditions, including pulse width, repetition rate, and peak power of the Raman laser. This parametric study was performed to determine threshold conditions required to generate visible marks. Moreover, we confirm superiority of the pulsed coagulation scheme compared to a previous paradigm that utilized a continuous-wave (CW) light source (380 mW at 1.48-?m wavelength). The pulsed operation allowed us to generate a mark even with a single short pulse of <100 ?s width, suggesting the possibility of fast registration or patterning without stationary irradiation over several seconds as needed by the previous CW laser operation.

8938-24, Session 5

Optical fiber spectroscopy measures perfusion of the brain in a murine Alzheimer's disease model

Hyung Jun Ahn, The Rockefeller Univ. (United States); Sidney Strickland, Rockefeller Univ. (United States); James Kreuger, Daniel S. Gareau, The Rockefeller Univ. (United States)

Optical fiber spectroscopy is a versatile tool for measuring diffuse reflectance and extracting absorption information that can noninvasively quantify the presence of chromophores such as oxy-hemoglobin and deoxy-hemoglobin in tissues. We report on adaptation of our previously developed fiberoptic spectrometer to measure saturation in the brains of mice. We used Tg6799 mice (Jackson Laboratory), which are transgenic mice expressing five different familial Alzheimer disease associated mutations in the human APP and presenilin 1 genes. This enhanced the onset of amyloid pathology, which is readily detectable at 2 months of age. Our methods include sedated animal restraint and scull thinning to enable the spectroscopic measurement. Diffuse reflectance spectra are then iteratively fit (least squares) as expected from homogenous scattering and a variable amount of oxy- and deoxy-hemoglobin. We show for two male mice, significant decrease in oxygen saturation and for one female mouse, reduces saturation that was not statistically significant. Ongoing work focuses on developing a cannulas fixture that allows measurement in awake, behaving animals.

8938-25, Session 5

Optical coherence tomography envelope statistics methodology to assess temperature changes in tissue mimicking fluids

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Several therapies make use of a hypo or hyperthermia tissue environment to induce cell death in both benign and malignant tumors. Current progression in optical technologies, such as optical coherence tomography (OCT) and fiber bragg gratings (FBG) sensors, could potentially provide viable information to explore the response of tissue when these temperature induced treatments are implemented. Studies were conducted with phantoms fabricated with polystyrene microspheres and glycerin to observe any relationship between the pixel intensities of the OCT images and their concurring envelope statistics, which mimic the optical properties of tissue. OCT images were taken of the FBG temperature monitored regions at 5°C intervals from 25°C to 60°C. The four probability distribution functions (PDF), Rician, Rayleigh, Normal and Generalized Gamma were used to investigate OCT envelope statistics as the temperature was altered. Using the Kolmogrov-Smirnov goodness of fit test it was determined the Generalized Gamma was the best fit. The scaling and shape parameters associated with the Generalized Gamma PDF were used to quantify the OCT envelope data to identify temperature changes within the tissue mimicking media. Apart from the

PDFs, the OCT speckle de-correlation at the varying temperature were also measured and quantified to detect the microspheres response to temperature changes. Initial results are very promising with future research focused on extending this methodology to monitor relative temperature changes in tissue during therapy. Clinical utility can be achieved if these optical techniques are used evaluate the temperature-derived biological response of tissue and provide a feedback mechanism to improve procedural efficiency.

8938-26, Session 5

Bio-functionalized hollow core photonic crystal fibers for label-free DNA detection

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The unique manufacturing technology of Hollow Core Photonic Crystal Fibers (HC-PCFs) allows for the precise tuning of the optical properties by changing the size, shape and position of the cladding holes. In addition to enhancement in light matter interactions, the hollow core fibers also offer large surface-to-volume ratios for experiments in which reactions of surface-bound sample species are of interest. These special properties have led to the development of several applications in the fields of optical communications, high power lasers and optical biosensing.

In this work, the bio-functionalization of inner surfaces of all silica HC-PCFs has been investigated. The approach is based on layer-by-layer self-assembly Peptide Nucleic Acid (PNA) probes, which is an oligonucleotide mimic that is well suited for specific DNA target recognition, allowing the recognition of specific sequences of DNA. Two kinds of HC-PCFs have been considered: a one dimension photonic Bragg fiber and a two dimension photonic hollow core (HC-1060) fiber. After spectral characterization and internal surface functionalization using PNA probes, genomic DNA solutions from soy flour were infiltrated into the fibers. The hybridization of the complementary strand of target DNA increased the optical thickness of the silica internal surface. This led the generation of surface modes, resulting in a significant modulation of the transmission spectra. Hence, the possibility to realize sensing for biological applications could be easily suggested

8938-27, Session 6

Efficacy of pressure relief maneuvers in spinal cord injury patients: a clinical study

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Pressure reliefs are recommended to wheelchair bound individuals to control and minimize skin damage occurring due to prolonged pressure on an area of the skin. To date recommendations on duration and intervals between pressure reliefs is not clear. Recent studies have shown a relationship between reduction in tissue perfusion and oxygenation due to pressure and skin pathophysiologic changes.

We have developed a fiber-optics probe that allows measurement of oxygenation in addition to perfusion in real time; this low profile probe can be utilized while sitting and during pressure reliefs. At the same time we are able to follow each individual movements on his or her wheelchair using a pressure mat.

We have conducted a clinical trial at MedStar National Rehabilitation Hospital monitoring oxygenation, perfusion, and pressure changes in sitting and during pressure reliefs on individuals with spinal cord injury. The overriding goal of this project was to develop the evidence base for clinical recommendations on pressure reliefs. Results of the study will be presented.

Feedback on desired frequency and duration of pressure reliefs is ultimately given to each patient.

8938-28, Session 6

Towards mid-infrared fibre-lasers: comparison of Ga- and In-containing, rare earth doped, selenide chalcogenide glasses and optical fibres

Hesham Sakr, Zhuoqi Tang, David Furniss, Lukasz Sojka, Nabil A. Moneim, Emma Barney, Slawomir Sujecki, Trevor M. Benson, Angela B. Seddon, The Univ. of Nottingham (United Kingdom)

Chalcogenide glasses are promising materials for mid-infrared (IR) applications i.e. 3-25 μm wavelength. They have low phonon energy and hence exhibit high mid-IR transparency enabling them to be promising candidates for mid-IR fibre lasers for applications in medical laser surgery and as pumps for mid-IR supercontinuum generation for molecular sensing, including medical sensing applications. The quality of the fibre is key to obtaining the highest possible optical intensity with the lowest optical loss. Gallium is considered one of the popular additive elements to the chalcogen group glasses, i.e. S, Se and Te, due to its ability to aid dissolution of the rare earth ion in the glass, increase the fluorescence lifetime and is supposed to complex the rare earth ion. On the other hand, gallium has a low melting point that results in liquid-state batching which causes some complications. The idea of replacing gallium (Ga) with indium (In) would avoid this; moreover both elements share the same Group in the Periodic Table and hence have similar chemical behaviour. Indium has the advantage of being heavier and hence enables lower local phonon energy for the rare earth ion that can provide better fluorescence behaviour of the lasers. The aim of this work is to replace Ga with In in Ge-As-Se glasses. A series of rare earth ion doped Pr3+-Ge-As-In-Se chalcogenide glasses is prepared and characterised. A comprehensive comparison of the glass behaviour, including light absorption, glass stability, glass emission, fibre drawing and fibre loss measurements, with Ga-based chalcogenide glasses is provided.

8938-29, Session 6

Towards mid-IR supercontinuum generation: Ge-Sb-Se mid-infrared step-index small-core optical fibre

Jessica H. Butterworth, Dinuka Jayasuriya, QingQuan Li, David Furniss, Nabil A. Moneim, Emma Barney, Slawomir Sujecki, Trevor M. Benson, Angela B. Seddon, The Univ. of Nottingham (United Kingdom)

In the 21st century, cancer has become a common and feared illness. Early detection is crucial in curing patients, yet current diagnostic tests depend upon the skill of a doctor and histologist for recognition of the cancerous cells. Therefore it is necessary to develop a medical diagnostic system which can analyse and image tissue instantly, removing the margin of human error and with the additional benefit of being minimally invasive.

The molecular fingerprint of biological tissue lies within the mid-infrared

(IR) region of the electromagnetic spectrum, 3-25µm wavelength. This can be used to determine a tissue spectral map and provide information about the absence or existence of disease, potentially in real-time and in vivo1. However, current mid-IR broadband sources are not bright enough to achieve this. One alternative is to develop broadband, mid-IR fibre, supercontinuum generation (SCG). Chalcogenide glass optical fibres have the potential to provide such mid-IR (SCG). A popular chalcogenide glass fibre type is based on Ge-As-Se. For biomedical application it is prudent to avoid the use of arsenic, on account of its toxicity. This paper investigates replacing arsenic with antimony, towards Ge-Sb-Se small-core optical fibres for SCG. Physical properties of candidate glass pairs are investigated for glass stability via differential thermal analysis etc. and fibre loss measurements of associated fibres are assessed. These results are compared to analogous arsenic-containing chalcogenide glasses and optical fibres and conclusions are drawn focusing on whether there is potential for antimony chalcogenide glass to be used for SCG for mid-infrared medical diagnostics.

1 AB Seddon, A prospective for new mid-infrared medical endoscopy using chalcogenide glasses, Int. J. Appl. Glass Sci 2 [3] 177–191 (2011).

8938-30, Session 6

Exposed core microstructured optical fiber surface plasmon resonance biosensor

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Surface Plasmon Resonance (SPR) scattering offers significant advantages compared to traditional reflectivity measurements, essentially turning a non-radiative process into a radiative one. Recently, we have shown that SPR scattering can be used in an optical fiber, enabling higher signal to noise ratio, reduced dependence on the metallic thickness as well as the unique capability of multiplexed detection with a single fiber. Here we report a novel SPR scattering based sensor fabricated based on an exposed-core silica Microstructured Optical Fiber (MOF). This MOF presents a structure with a relatively small core (Ø=10 ?m), exposed along the whole fiber length. This exposed core MOF allows for fabrication of SPR supporting metallic thin films directly onto the fiber core offering the new prospect of exploiting SPR in a waveguide structure that supports only a relatively small number of guided optical modes, with a structure that offers ease of fabrication and handling. A thin silver film of 50 nm thickness was deposited onto the fiber core by thermal evaporation. The significant surface roughness of the prepared metallic coatings facilitates strong scattering of the light wave coupled into the surface plasmon.

Performance characteristics of the new exposed core fiber sensor were compared to those of a large bare core silica fiber (Ø=140 ?m). Although sensitivity of both sensors was comparable (around 2500 nm/RIU), full width at half maximum (FWHM) of the SPR peaks for the new exposed core fiber sensor decreased by a factor of 3 offering an significant enhancement in the detection limit of the new sensing platform in addition to the prospect of a sensor with a lower detection volume.

8938-31, Session 7

Hollow fiber based SERS probe for analysis of biological molecules

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A Flexible fiber probe for surface-enhanced Raman scattering (SERS) is proposed and fabricated. The probe is fabricated by attaching a ball lens in which gold nanoparticles is formed on the surface at the distal end of a hollow optical fiber. Two different methods are used to form the gold nanoparticles. In the photochemical deposition, easily fabricated probe indicated good sensitivity, but low reproducibility. On the other hand, in the sputtering method, higher intensity is achieved constantly because an optimal forming condition can be strictly controlled. The measurement of biological molecules using fabricated SERS active probe is also demonstrated.

8938-32, Session 7

Temperature controlled laser bonding of incisions in tissues through a single compound fiber

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The advances of modern medicine towards non-invasive surgery have encouraged researchers to design novel systems for fiber-optic laser bonding of tissues as an alternative to the traditional use of sutures. An accurate fiber-optic system may also improve surgeons' ability to bond thin and delicate organs in ophthalmology, microsurgery and in other medical disciplines. The current study introduces a novel system for laser bonding of incisions in tissues.

In the past we have developed a fiber-optic system that was based on two mid-IR fibers. One was used to transmit CO2 laser to the tissue, and the other transmitted blackbody radiation from the tissue onto a detector. Recently, we have managed to do both through a single Infrared fiber. We have also developed a new system based on a 1.9µm semiconductor disk laser. The fiber-optic setup was based on a compound fiber that consisted of a central silica core and AgClBr cladding.

The novel systems were researched and characterized. They were used for in vitro bonding of cornel incisions in freshly enucleated bovine eyes. The use of 10.6µm or 1.9µm wavelength ensured that the laser beam penetrated exactly to the right extent to generate a strong bonding without endangering the retina. These preliminary experiments with the systems clearly demonstrated their advantages. The bonding was stronger, the systems were easier to use and the temperature measurement was much more accurate. These advantages will probably make it easier for this kind of systems to be incorporated in the operating rooms in the near future.

8938-33, Session 7

Influence of optical fiber bundle parameters on the transmission of laser speckle patterns

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Laser speckle imaging (LSI) techniques provide important functional information about tissue perfusion and mechanical properties. To perform LSI in vivo, laser speckle patterns are transmitted via coherent optical fiber bundles incorporated within a small diameter endoscope. Mode coupling due to inter-fiber cross-talk in fiber bundles can result in erroneous speckle statistics and therefore reduces the efficacy of LSI analysis. In this study, we analyze the influence of multiple fiber bundle parameters on the temporal statistics of laser speckle fluctuations and design a new optical fiber bundle to considerably reduce the modulation of transmitted speckle patterns caused by mode coupling between and within multi-mode fibers. We have applied a theoretical model based

on coupled mode theory and have calculated the coupling coefficients between all modes of adjacent fibers. We have analyzed the influence of fiber core size, core spacing, numerical aperture (NA) and variations in core size, on coupling between modes of individual fibers and speckle intensity modulation. The influence of bending and twisting of fiber bundles on mode coupling and speckle pattern transmission are also considered. Our results show that large NA, reduced number of propagating modes and large core-to-core separation are essential to diminish fiber mode coupling and improve temporal speckle statistics. The analysis of the speckle intensity autocorrelation of time-resolved speckle frames showed that a fiber bundle with 3?m core size, 8?m core spacing and 0.40 NA permits reliable speckle transmission to conduct endoscopic LSI at 690 nm.

8938-34, Session 7

High-resolution fiber-optic bundle microscopy based on dithered spatial compounding

Gyeong Woo Cheon, Jaepyeong Cha, Jin U. Kang, Johns Hopkins Univ. (United States)

Coherent fiber bundles with high core density are highly effective tools for high-resolution endomicroscopy. Nevertheless, fiber bundles inevitably have un-imaged regions that occur between adjacent cores. This results in structural artifact known as pixelation effect an decreased resolution. Many kinds of image processing techniques have been introduced to remove this pixelation artifact such as frequency domain filter and Gaussian filter. However, these methods are fundamentally limitated because they all use the information of adjacent pixels to fill in the un-imaged area. To overcome this problem, we introduce a dithered spatial compound imaging method to eliminate the pixelation artifact and eliminate the un-imaged areas. The method uses multiple frames taken with small deviation of position (i.e, intra-pixel dithering). Some parts of these images include information which is devoid of in other images. The total amount of information increase as more images are compounded and the significant improvement of resolution in the final images are achieved. At the same time, the duplicated parts among these images can be averaged to improve SNR ratio. To achieve these improvements, a sophisticated registration algorithm to precisely align the image is needed. The pixelation artifact is troublesome again in registration process because its structural artifacts are strong features shared with whole images. However, we can solve this problem by using reference image and divide the sample images into two parts: effective and ineffective regions. We can then use the effective regions for registration. We used USAF target to evaluate our method and the result shows that SNR and resolution can be significantly increased.

8938-35, Session 7

Multi-channel surface plasmon sensor using a unique H-cross-section optical fiber

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We present theoretical and experimental study of a novel structure for a multi-channel surface Plasmon resonance (SPR) bio-sensor using a specially designed H-cross-section fiber. The flat bottom of the channeled fiber facilitates metal coatings, allows the construction of a multi-channel SPR sensor with reduced complexity and sensitivity to fiber deformations or bending, and provides more flexible sensing area. The fiber is birefringent with a core diameter of 6.63 ? 5.72 µm. The sensor has two channels using the flat side which is closer to the core where the cladding has been removed completely by wet HF etching. The two layers are coated with 2 nm chromium, 50 nm of gold. The second channel is coated with additional 20 nm over-layer of Tantalum Pentoxide (Ta2O5). The thickness of Ta2O5 is used to tune the SPR wavelength to a desired value [1]. The two channels show different characteristics and distinctly different resonant wavelengths at which the guided mode couples directly to the surface Plasmon without the use of a phase-matching grating. The simulation results using the attenuated total internal reflection approach and the N-layer model [2] for a single mode propagating in the vertical axis shows that the first channel has a sensitivity of 3500 nm/RIU and a resolution of 2.8 ? 10-6 RIU while the second channel shows a sensitivity of 3250 nm/RIU and a resolution of 3 ? 10-6 RIU . This sensor allows another technique for discriminating biological samples, by providing a differential response.

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8938-36, Session 7

Micro sized implantable ball lens-based fiber optic probe design

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Microscopes employing fiber-optics have become an indispensable tool for brain activity detection in live animals. Nevertheless, several issues still remain to be solved. One is the invasiveness of the probe inserted into the deep brain areas with the risk of disrupting blood circuitry. Another is the lacking of focusing lens caused low collection efficiency of returning light from the brain tissue.

In this study, we present a novel design and fabrication of a micro-size implantable ball lens-based fiber optic probe, which is implanted in the skull and allows continuous monitoring of brain activity in freely behaving mice. A prototype uses a 500-micron ball lens and a highly flexible 200-micron core multimode fiber that are encased by 800-micron cubic polymers and 25-Guage stainless steel tube, respectively. This system allows repeated attachment of the fiber to the lens, which enables longitudinal studies on animal behaviors. We first simulated our design based on geometrical optics and evaluated the performance using several types of brain tissue, consisting of fluorescence probes such as GFP, GCaMP3 calcium indicators. Measured working distance is approximately 1-mm, which is enough to detect neural activities from cortical and cerebellar tissues of mice brain. This technology has the potential to enable simultaneous brain activity monitoring in multiple regions using spatially multiplexed imaging.

8938-37, Session 8

CP-OCT sensor guided SMART micro-forceps

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Surgeons are not able to hold their hands perfectly still during microsurgery due to their physiological tremor. Even the most stable hands make nearly invisible movements on the order of 50-100 microns within 0-15 Hz. Micro-forceps are one of the frequently used surgical tools to grasp or divide the wound tissue into thin layers for microsurgery. Here, a handheld Smart Micromanipulation Aided Robotic-surgery Tool (SMART) micro-forceps is demonstrated by integrating a fiber-optic common-path optical coherence tomography (CP-OCT) sensor into the micro-forceps. This forceps design could significantly improve to cancel unwanted hand tremor during a moment of a grasping by introducing thin-wall squeezing part free design. Also, dual OCT distance sensing scheme is implemented at the front and back part of the SMART handheld device. Front sensor has a ball lens fiber-optic sensor to

measure the distance from the sample surface and back sensor functions as motor encoder to get the current position. The basic grasping and peeling functions of the micro-forceps are evaluated in dry phantoms and in a biological tissue model. As compared to freehand use, targeted grasping and peeling performance assisted by active tremor compensation, significantly improves micro-forceps user performance. This comparison is also demonstrated by 3D OCT assessment. This design improves previous SMART micro-forceps performance, particularly in tremor reduction at the moment of the grasping.

8938-38, Session 8

Biconically tapered fiber optic dip probe for rapid label-free immunoassays

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A biconically tapered optical fiber (BTOF) is a simple and cost-effective refractive index sensor based on modal Mach-Zehnder interferometry. For biosensing applications, the surface of the taper is typically functionalized with antibodies specific to an antigen to be detected. The antibody-antigen reactions create a biological nanolayer on the surface of the tapered region, which modifies the waveguide structure resulting in a phase difference between the propagation modes. As a result, spectral shifts are measured in the transmission spectrum of the sensor. Unlike biosensors that work based on fluorescent labels, the data can be recorded in real time enabling the study of the kinetics of antibody-antigen binding. In all previous studies, BTOFs based on Mach-Zehnder interferometry were reported in straight geometry, which cannot be used as a dip probe that can be immersed in solutions because of geometrical limitation. In addition, when there is a temperature increase, the substrate of the taper expands and the tensile stress on the sensor increases leading to the possibility of breakage and increased temperature sensitivity. We report U-shaped biconically tapered optical fibers (BTOF) as dip probes for label-free immunoassays. The tapered regions of the sensors were functionalized by immunoglobulin-G (Ig-G) and tested for detection of anti-IgG at concentrations of 0.5, 5.0, and 50 microgram/mL. The limit of detection was estimated to be less than 0.5 microgram/mL with low temperature sensitivity. Utilization of the rate of the sensor peak shift within the first few minutes of antibody-antigen reaction is proposed as a rapid detection method.

8938-39, Session 8

Application of Ball-lens Hollow Fiber Raman Probe For Studying Anorectal Prolapse

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Our Raman probe that is called as ball-lens hollow fiber Raman probe (BHRP) had been proved possessing capability to detect the biochemical alteration within biological tissue. Whether BHRP has high capability and sensitivity in diagnosing the biochemical changing of tissue or not, mouse's normal rectal and anorectal prolapse (AP) were decided to be used as a model for this non invasive method. This AP is Azoxymethane-induced mouse's anorectal prolapse. Main outcome of Ball-lens hollow fiber Raman probe (BHRP) will be potential for non-invasive method in tumor diagnosing. BHRP spectra obtained were high quality and allowed analysis of their differences between normal (control group) and AP. After spectral acquisition and comparison with corresponding images from hematoxylin/eosin-stained section observation used to make the histopathologic diagnosing, BHRP detected that azoxymethane-induced tumor, mice's tumor on anorectal prolapse have some differences within the region of moiety of protein (i.e. collagen), DNA, and lipid, then following with the alteration of amide I and amide III compared with the normal rectal tissue. BHRP discriminate normal tissue and AP in the real-time.

8938-40, Session 8

Chalcogenide microstructured optical fibers for chemical sensing

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Chemical bonds of most of the molecules vibrate at a frequency corresponding to the near or mid infrared field. It is thus of a great interest to develop sensitive and portable devices for the detection of specific chemicals and biomolecules for various applications in health, the environment, national security and so on. Optical fibers define practical sensing tools.

Chalcogenide glasses are known for their transparency in the infrared optical range and their ability to be drawn as fibers. Such optical fibers can transmit light from 2 to 20 µm depending on the composition of the glass constituting the fiber. They are consequently good candidates to be used in biological/chemical sensing.

Different types of fiber can be used: single index fibers or microstructured fibers. Besides, in recent years a new configuration of microstructured fibers has been developed: microstructured exposed-core fibers. This design consists of an optical fiber with a suspended micron-scale core that is partially exposed to the external environment. This configuration has been chosen to elaborate, using the molding method, a chalcogenide fiber for chemical species detection. The sensitivity of this fiber to detect molecules such as propan-2-ol and acetone has been compared with those of single index fibers. Although evanescent wave absorption is inversely proportional to the fiber diameter, the result shows that an exposed-core fiber is much more sensitive than a single index fiber having a twice smaller external diameter.

8938-41, Session 8

Bending compensation for multimode fiber based endoscopes

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Imaging inaccessible structures has become possible by modern endoscopy. Recently there has been an increasing interest of using a single multimode fiber (MMF) for both light delivery and collection.

Many techniques have been developed to compensate for modal scrambling inherent to MMFs, enabling imaging through them. They thus provide lensless imaging with a sub-micrometer resolution and are a suitable solution to further miniaturize endoscopes.

The main drawback of imaging through a multimode fiber is that it is highly sensitive to bending. When the fiber is bent, the modal dispersion cannot be compensated anymore. One approach is to use a completely rigid multimode fiber endoscope that is calibrated once for one specific spatial conformation of the fiber. However, for some applications, a flexible or semi-flexible endoscope is necessary.

We present a solution to dynamically compensate the fiber bending while focusing light through a multimode fiber by using a virtual beacon for digital phase conjugation. We experimentally implement it by recording at the fiber tip, a hologram of a spherical wave generating a virtual focus point. We also show that this virtual beacon source can be used

to uniquely determine the fiber spatial configuration by comparing the speckle pattern originating from it with previously recorded speckle patterns. The fiber can then be characterized without having access to its distal tip, in a reflection configuration. These results pave the way to many new applications of high-resolution lens-less endoscopes based on digital scanning through semi-flexible optical multimode fibers.

8938-42, Session 8

Label-free biochemical characterization of sperm cells using Raman microscopy

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X and Y chromosome-bearing sperm cells sorting has been of great interest, especially for breeding programs. Several methods have been proposed for modifying mammalian semen to increase the relative percentage of X- or Y-sperm in a semen sample, and thereby achieve a greater likelihood of female/male offspring. However, the proposed techniques still has problems in terms of sample damage and measurements reproducibility.

The aim of the present study is to evaluate possible differences in term of biochemical composition of the sperm head between X and Y chromosome-bearing sperm by Raman spectroscopy.

Raman spectroscopy is a non-invasive technique based on the inelastic scattering of laser photons upon interaction with the sample molecules. It allows the characterization of the properties and structure of the molecules from their stretching and bending vibrational transition [1,2].

In this paper, we present a Raman spectroscopy-based method for selective and sensitive discrimination of X and Y bovine sperm cells. More precisely, we acquired and compared 300 Raman spectra of X and Y spermatozoa from three different donors. We demonstrate that a spectroscopic signature, combination of the spectral component in the sperm (DNA, proteins, lipid etc.), can be used to identify the biochemical components and differences between X and Y sperm cells. Additionally, a multivariate statistical analysis (PCA, Principal Component Analysis) has been used to distinguish X and Y bovine sperm cells based on single cell Raman spectra.

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8938-43, Session 8

Large-core tube-leaky waveguide for delivery of high-powered Er:YAG laser

Shun Kobayashi, Takashi Katagiri, Yuji Matsuura, Tohoku Univ. (Japan)

We present a tube-leaky fiber for delivery of Er:YAG lasers light. The tube-leaky fiber consists of only dielectric thin-film tubing and light is confined in the airy core when the film thickness is properly chosen for target wavelength. Transmission properties of the fibers are derived by using a ray optic method and designed the optimum wall thickness for the Er:YAG laser wavelength of 2.94 micron. In fabrication of the tube leaky fiber, we use a microstructural tube made of glass that is transparent at the laser wavelength. The central bore and surrounding glass thin layer that function as a tube-leaky fiber are held by microstructure support. We fabricate a large-core fiber for delivery of high power medical lasers by stack-and-draw method and we use borosilicate-glass as a fiber material for easy and low cost fabrication. Fabricated fibers have a diameter over 400 ?m and from the loss measurements for Er:YAG laser, and the fibers deliver laser light with a transmission loss of 0.85 dB/m that is comparable to 0.7 dB/m of conventional hollow-optical fibers. The fibers withstand transmission of high energy laserpulses of order of 80-120 mJ. We confirm that these energies are enough to ablate biological tissues in surgical operations through the experiment using biosamples.

8938-44, Session PSun

Full-field optical coherence tomography system implemented with fiber-optic components

Eun-Seo Choi, Seung Suk Lee, Joo Ha Kim, Chosun Univ. (Korea, Republic of)

We have presented full-field optical coherence tomography(FF-OCT) system implemented with fiber optics rather than bulk optics. Usually FF-OCT system illuminates large area at once while conventional OCT irradiates light to single focal point. From these reason, light guidance with single fiber waveguide is not proper and fiber-optic components is not dealt in the system implementation. In this paper, we demonstrate FF-OCT system consists of fiber-optics, where fiber coupler and fiber circulator were used to perform the function of beam splitting and optical delay line. Each arm of fiber coupler acts as reference arm and sample arm. Fiber-optic collimator and metal-coated mirror mounted on translator in the reference arm could adjust optical path length. Separated beam after the fiber coupler was combined after bulk beam combiner, where beam size is expanded and illuminated to sample. The larger size beam reflected from sample was interfered with reference beam, which experienced optical delay in the reference arm. The utilization of fiber-optic components could provide merits such as easiness in optical alignment and reduction of sensitiveness to external vibration and perturbation. For the check of the system feasibility, biomedical and non-biomedical tomographic imaging was obtained and analyzed.

8938-45, Session PSun

Empiric model for identification of Laser tissue soldering process completion using IR-spectroscopy

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Laser Tissue Bonding (LTB) refers to a bonding method where a laser beam heats the approximated edges of an incision. The method work well if the approximated edges are heated to T ~60ºC for approximately t=10 sec. This bonding method has significant advantages over conventional methods, based on sutures or chemical glues. However, in order to obtain reliable and repeatable bonding one must carefully control both the bonding temperature T and the bonding duration t.

In the past, we developed a non- contact fiber-optic infrared laser bonding system. This system heats a spot on an incision, and it monitors and controls the temperature of the spot. The surgeon keeps heating a spot for a few seconds and then moves the spot to a neighboring location. But, the system does not provide any indication that the edges of the tissues were indeed strongly bonded. There is a need to develop a method for determining the “end point” of the bonding procedure.

We studied the laser bonding of incisions in vitro in calf corneas. In every experiment each spot was heated to T for time t. During the bonding process, the system measured the spectral signal (in the mid-IR) emitted by the tissue. At the end of the bonding process we measured the force needed to separate the bonded tissues, and determined whether the edges were strongly bonded or not. Using a Data Learning Algorithm, the system learned to map two separate outcomes: Bonding Completed

or Incomplete Bonding. The system thus learned to use the information obtained from spectral measurement to determine in real time the “end point” of the process. The system achieved a prediction level better than 90% (bonding completed / not completed)

8938-46, Session PSun

Surface plasmon resonance based fiber optic urea sensor using ITO and enzyme

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The determination of urea in blood and urine is an important measure of the functioning of kidney in human body. It is produced by the liver and transported to the kidneys via blood for excretion. Change in urea concentration in blood is the result of malfunctioning of kidney and liver. Thus the estimation of urea concentration is very important for the diagnosis of such diseases.

In SPR technique, electron charges perform coherent oscillations at the metal–dielectric interface, which are called as surface plasmons. These are excited by a TM polarized light and the fields associated with them have its maxima at the interface and decays exponentially in the metal as well as in the dielectric medium.

In this paper we report the fabrication and characterization of a surface plasmon resonance based fiber optic sensor for the detection of urea in a liquid. The probe is fabricated by coating two layers namely of ITO and the enzyme, urease, over about 1 cm length of the unclad core of an optical fiber. The wavelength interrogation method is used to characterize the sensor. It is observed that the resonance wavelength decreases as the concentration of urea increases. The sensor operates in the urea concentration range of 0–160 mM, which is close to the physiological range of urea in blood and hence can be used in medical sciences.

8938-47, Session PSun

Cost-effective optical coherence tomography spectrometer based on a tilted fiber Bragg grating

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A high performance spectrometer utilizing a tilted fiber Bragg grating (TFBG) as integrated dispersive element for biomedical application is demonstrated. Based on the 45° UV-written PS750 TFBG a compact refractive spectrometer with 1.83radiant/µm dispersion and 0.1 numerical aperture was set up and tested. Featuring a 15mm long active region a spectrum is projected through a cylindrical lens for vertical beam collimation and successively by an achromatic doublet onto a 2D CCD array. The spectrometer was fiber-optically connected to a Michelson interferometer with an optional motor-driven sample mirror arm to interferometrically verify the spectral resolution and the corresponding depth dependent fringe loss to -3dB/0.4mm. Since the sensitivity was limited by the chromatic error of the cylindrical lens, the size of the focusing lens and the instability of the fiber mount a second spectrometer with a USB line-camera (3648 pixels, 8µm pixel pitch) was devised to span a 5.5mm depth scan range in air. The fine adjustable and rigid device was numerically optimized for broadband operation to maximize throughput and minimize chromatic error. Covering 740nm to 860nm the device was connected to different scanning heads and an electronic acquisition and control unit. High resolution tomograms of ophthalmic and dermal samples obtained by the frequency domain OCT-system will be presented. The compact size and dense integration as a simple fiber-optic mount to existing optical-electronic infrastructure overcomes mechanic limitations of bulk systems, enables miniaturization at reduced costs and has the potential to extend the field of application for OCT-systems in biology, medicine and technology.

8938-48, Session PSun

Blood pH optrode based on evanescent waves and refractive index change

Krister Hammarling, Mid Sweden Univ. (Sweden); Jöns Hilborn, Uppsala Univ. (Sweden); Hans-Erik Nilsson, Mid Sweden Univ. (Sweden)

A fiber optic pH-sensor for blood measurements has been developed, by combining a standard multimode silica fiber with a nontoxic biocompatible pH sensitive Poly (?-amino ester). There is an increasing demand for sensors that can measure the pH level in an accurate and simple way, and also for long term monitoring. The optical sensor principle is based on evanescent wave absorption for measuring the refractive index change in the cladding. This polymer has the ability to expand or contract in response to different pH values and thereby change the overall polymer volume, causing a change in the refractive index and the transmission properties of the optical fiber. The Poly (?-amino ester) can be chemically tailored for a high pH response in different pH ranges and also offers the possibility to use of a wide selection of light sources from the UV to the near infrared spectral range. A standard multimode fiber (105µm core and 125 µm cladding) was used to guide the light and act as the sensing element. In the middle of a short piece of fiber, 5 cm of the coating was stripped away. There, the silica cladding was chemically etched down to the 105 µm core and replaced with a thin layer of the pH-sensitive poly (?-amino ester). Depending on the molar ratio between the poly (?-amino ester) components, 1.4-Butanediol Diacrylate (BDDA) and Piperazine (PIP), the region for a high sensitivity to a specific pH range can be tailored.

8938-49, Session PSun

Microfluidic on optical fibers: towards a new kind of fluorescent biosensor

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In recent works, the behavior of droplets moving along vertical treads due to gravity was studied. It appeared that the droplet can be stopped by encountering a horizontal fiber depending on droplet volumes and fiber characteristics. On the basis of this behavior and by replacing treads by two crossed optical fibers, it is possible to combine fluidics and optics to develop a new kind of fluorescent sensor.

In our work, the intersection between two crossed optical fibers is used as the basic unit of an original optofluidic biosensor. These two optical fibers are used as droplets carriers: one for probe molecules and the other one for target species. The fiber’s junction catches the droplets and act as a reaction center. The main advantage of using optical fibers resides in their ability to propagate and collect light to and from the droplet localized at the fiber’s crossing. This optical fiber configuration can therefore allow the study of biological interactions using fluorescent labels.

This new and versatile detection scheme was validated on a calcium indicator where ions detection is accomplished by using a dye, Oregon green Bapta-2, that has a Ca 2+ recognition group as well as an entity exhibiting fluorescence. A FRET recognition event, between Rh-Con A and FITC-Dextran, was also investigated to detect glucose. Finally, a prototype of a multiplexing device, composed of several juxtaposed fibers’ junctions, was developed.

8938-50, Session PSun

Dental caries diagnosis system with UV fiber bundle lighting and camera

Joo Beom Eom, In Hee Shin, Jae Seok Park, Hyeong Ju Park, Byeong-Il Lee, Korea Photonics Technology Institute (Korea, Republic of)

Dental caries diagnosis system has been made with a fiber bundle lighting and camera module. By simply aligning a UV LED (405 nm) directly connected fiber bundle, a compact lighting for dental fluorescence imaging could be implemented. To achieve a fluorescence image and dental caries diagnosis, the proper optical filter, camera module, UV fiber bundle lighting and software were investigated. Experimentally, an optical insertion loss of the fiber bundle of up to 0.7 dB and resolution of the dental caries of up to 2 mm were achieved. The combination efficiency between fiber bundle and UV LED was about 15 %. With a fiber bundle lighting and camera module, a dental caries diagnosis system was implemented and used demonstrate the feasibility as the dedicated diagnosis system for dentistry

8938-51, Session PSun

Optimized surface enhanced Raman spectroscopy (OSERS) using nanosphere lithography phase I: flat substrate characterization and optimization

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Surface Enhanced Raman Spectroscopy has been widely employed to enhance Raman signals but, it is limited in terms of reproducibility. This study investigates the optimization paradigms to be employed towards improving the reproducibility and predictability of SERS by employing a combination of theoretical modeling and experiments. We investigate methods to allow fine-tuning the formation of nanostructured geometries to obtain tailored plasmon-resonant ranges while economizing the design procedure. Substrates were prepared using Nanosphere lithography (NSL) where different sizes of Polybead microspheres (Polysciences Ltd. ranging from 0.2 - 1 micron) were used to prepare a mask through self-assembly on the surface of the substrates. Following the mask creation, gold was sputtered (using Denton Vacuum Desk IV) onto the substrates and then, the micro-spheres were removed through sonication. The surface of the substrates was characterized using SEM and the dimensions of the fabricated nano-structures were calculated. The histograms of the sizes of the nano-structures were collected to comment on their reproducibility. A spectrophotometer (Shimadzu UV 3600) was used to characterize the Plasmon resonant ranges of the fabricated substrates and non-normal angles of incidence were also investigated. Enhancement factors were obtained for a few Raman active dyes (Rhodamine-6-G and Cyanin dyes) and molecules. The SEM images were binarized to allow for measurement of averaged size and shape of the nano-structures. A preliminary scattering model was developed in COMSOL and the near-field enhancements of the structures were investigated. These results would be employed towards preparing SERS sensors to be utilized towards bio-medical sensing.

8938-52, Session PSun

Optical fiber-based photomechanical molecular delivery system

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Molecular delivery system based on nanosecond pulsed laser-induced photomechanical waves (PMWs) has an advantage that enables endoscopic application by using an optical fiber. We previously developed a fiber-based molecular delivery system, in which a laser target consisting of a black natural rubber film as a laser absorbing material covered with an optically transparent polyethylene terephthalate disk for plasma confinement was attached to the output end of a 1 mm core diameter quartz fiber. However, there were problems associated with the large outer diameter (~2.7 mm) and rapid decrease in the available peak pressure with increasing pulse number. In this study, we developed a new fiber delivery system to overcome these problems. As a laser absorbing material, we used a cap-type silicone rubber containing carbon black, into which the fiber output end can simply be inserted. The fiber end surface can work to confine the laser-induced plasma. The outer diameter of the fiber system was reduced to ~1.4 mm. At an output laser fluence of 1.2 J/cm2, peak pressure of the first PMW pulse exceeded 40 MPa. For successive 10 laser pulses with the same target, decreasing rate of the peak pressure was 22 %, which was considerably lower than that with the previous fiber system (82 %), enabling irradiation with at least successive 30 laser pulses for the same target. With this fiber system, we attempted transfer of plasmid DNA encoding EGFP (enhanced green fluorescence protein) to rat skin as a test tissue, showing site-selective efficient gene expression.

8938-53, Session PSun

Self-referenced label free biosensors based on differential fiber optic interferometry

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The measurement of refractive index (RI) is an important tool for label free biosensing in biomedical applications [1]. In this context fiber optic sensors present a valuable solution due to, high sensitivity, small size, and capability for in-situ, real-time, and remote sensing. Several fiber refractometers for biosensors applications have already been proposed. Surface plasmon resonance (SPR), long period gratings (LPG) and etched fiber Bragg gratings (FBG) among others [2]. These probes have shown high sensitivity to RI. Nevertheless, they are also sensitive the influence of temperature and non-specific binding events that must be accounted for when very high sensitivity and specific detection of biological parameters is carried out. Therefore, the desirable situation is to have a system that can perform non ambiguous measurement while operating in complex media.

In this work a fiber optic interferometric system for differential RI based biosensors is presented. The system is based on a white light Mach-Zehnder configuration, with serrodyne phase modulation, used to interrogate two similar LPG based optical fiber sensors in a differential scheme. In the situation where both devices are functionalized being one active (sensor) and the other one passive (reference) it is possible to accurately measure the analyte of interest independent of non-specific binding events, bulk refractive index changes and temperature. Signal processing with low cost digital instrumentation developed in Labview environment allows a detectable change in refractive index of ?n≈ 2x10-6 [3]. The system was validated with the detection of trombin using an aptmer based probe as a model system for protein detection. The surface of the sensing fibres was cleaned with ethanol and dried with N2 and the aminated trombin aptamer (GGTTGGTGTGGTTGG) was immobilize by

physisorption using Poly-L-Lysine (PLL) as cationic polymer. The results obtained indicate the viability of truly self-referenced biosensors.

8938-54, Session PSun

Comparison of photoacoustic spectroscopy and Fourier-transform infrared attenuated total reflection spectroscopy on biofilm

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One of the challenges in biofilm detection using hyperspectral imaging technology is low specificity compared to other traditional techniques such as biological assay. To complement the rapid and wide area detection capability of hyperspectral imaging, we utilized two spectroscopic methods based on Fourier-transform infrared (FTIR) spectroscopy: the attenuated total reflection (ATR) and the photoacoustic spectroscopy (PAS). Not only does PAS directly measure absorption, it is also known to provide at least an order higher sensitivity compared to ATR - which instead measures transmission. In this study, we utilized a microelectro-mechanical system (MEMS) based PAS detector for measuring absorption spectrum of Pseudomonas aeruginosa biofilm, and compared the results with those using ATR. Due to the strong absorption spectrum of water in growth medium, the spectral signature of Ps aeruginosa biofilm was difficult to detect by both PAS and ATR. However, when the water content was decreased by drying and with higher bacterial concentration, PAS provided slightly higher sensitivity over ATR. Results for samples other than biofilm will also be discussed.

Conference 8939: Biomedical Vibrational Spectroscopy VIII: Advances in Research and Industry

BiOS SPIE Photonics West

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8939 Biomedical Vibrational Spectroscopy VI: Advances in Research and Industry

8939-1, Session 1

Analysis of virus infected cell by Raman spectroscopy and transmission electron microscopy.

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Raman spectroscopy is a promising tool for detection of virus infection in live cells. Raman spectroscopy is able to analyze composition of proteins and lipids in aqueous condition. It provides enough high spatial resolution for analysis of live cells. In the present study, we demonstrate its feasibility to observe dynamic reaction of the live cell infected by virus. Method Raman spectroscopy might open new era in future virus study. The Raman spectra of the adenovirus infected live cell (293 HEK) were obtained after 12, 24 and 48 hours after the infection and compared with the spectra of control cell. Obtained spectra were analyzed with principal component analysis (PCA) suggest that differentiation is possible. The band at 1640 cm-1 increases its intensity in the spectra measure 12 and 24 hours after the infection. In contrast, the spectra obtained 48 hours later show the changes in a band at 1301 cm-1 which seems arising from lipids. The result suggested that Raman spectroscopy can detect the infection much earlier than the conventional immune-staining method. The infection of the virus was also examined by immune-staining, transmission electron microscope (TEM) and gas chromatography to analyze the origin of the alteration. The conventional techniques for detection of virus infection rely on immunology or gene expression, such as fluorescent protein and require the target virus to detect in advance to the analysis. In contrast, the Raman detection observes the reaction of the cell itself; it is not requiring specifying the type of virus.

8939-2, Session 1

Distinction of tumor-derived vesicles from normal vesicles by Raman microspectroscopy

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Background: Cells release vesicles, also called exosomes or microparticles, which are spherical particles containing a phospholipid bilayer. These vesicles are abundantly present in human blood and it is becoming increasingly clear that they contribute to many homeostatic processes, for example coagulation and intercellular signaling. Therefore, vesicles are a potential biomarker for disease. However, due to their small size (30 nm – 1 ?m), vesicle detection is a major challenge. The cellular origin of vesicles is usually established by fluorescent antibody labeling, which is laborious and involves practical problems. We have applied Raman microspectroscopy to distinguish tumor-derived vesicles from normal vesicles in solution without labeling.

Methods: Tumor-derived vesicles were isolated from a human pancreatic adenocarcinoma cell line and platelet and erythrocyte vesicles were isolated from blood bank concentrates. Vesicles were isolated using differential centrifugation and analyzed by transmission electron microscopy, nanoparticle tracking analysis, and Raman microspectroscopy. For Raman microspectroscopy, a 100 mW krypton ion laser operating at a wavelength of 647 nm was focused to a probe volume of 1 fL, which overlaps with the dimension of vesicles. The Stokes shift from light scattered by optically trapped vesicles was measured using a spectrograph dispersing in the range 646–849 nm.

Results: The Raman spectra of single optically trapped vesicles showed spectral transitions characteristic of phospholipids. Moreover, single optically trapped tumor-derived vesicles showed unique Raman transitions compared to platelet and erythrocyte vesicles.

Conclusions: For the first time, single tumor-derived vesicles were distinguished from normal vesicles without fluorescent antibody labeling using Raman microspectroscopy.

8939-3, Session 1

Identifying adult stem cells residing in the bulb area of hair follicles using micro-Raman spectroscopy

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Adult stem cells (ASCs) have been found amongst differentiated cells in many more tissues and organs than was once thought possible. These findings have opened up the possibility of using ASCs in a wide range of transplants. However identifying ASCs in tissue without damaging them is a major challenge. The objective of this study was to determine whether the minimally invasive method of micro-Raman spectroscopy (mRS) can be used to identify ASCs in situ. To test this idea, ASCs were identified in the bulb area of hair follicles within murine skin. Two sequential thin sections of each sample were used, one section in the sequence was stained for ASCs, and the other left unstained for mRS measurements. A Raman spectrum was acquired from a location within a cell of the bulb area by focusing 515 nm excitation light onto it with an x100 objective lens. A clear Raman spectrum was obtained in a few seconds. This was repeated around 40 times at locations distributed uniformly within each cell. Altogether spectra were obtained from 100 cells: 52 were assigned as putative ASCs and 48 as putative differentiated cells by their spatial correlation to stained regions in the matching thin section. The Raman spectra from each cell were averaged, and multivariate statistical analyzes were performed on these averages with a leave-one-out cross validation procedure. Using this method ASCs and differentiated cells could be separated with 98% sensitivity and 94% specificity. Changes in nucleic acids, lipids and proteins were evident between the two groups.

8939-4, Session 1

Differentiating the growth phases of single bacteria using Raman spectroscopy

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In this paper we present a longitudinal study of bacteria metabolism performed with a novel Raman spectrometer system. We have observed changes in bacteria metabolism, especially during growth phases, for different bacteria species, i.e.Escherichia coli and other

Enterobacteriaceae. Longitudinal study is possible with our Raman setup since the overall procedure to localize a single bacterium and collect a Raman spectrum last only 1 minute. Localization and detection of single bacteria is performed by means of lensfree imaging, whereas Raman signal (from 600 to 1800 cm-1) is collected into a prototype spectrometer that performs high light throughput (HTVS technology, Tornado Spectral System). Performing time-lapse Raman spectrometry during growth of bacteria, we observed variation in the net intensities for some band groups, e.g. amides and proteins. The obtained results clearly indicate that growth affects the Raman chemical signature. We performed a first analysis to check spectral differences and similarities. It allows distinguishing between lag, exponential and stationary growth phases. And the assignment of interest bands to vibration modes of covalent bonds enables the monitoring of metabolic changes in bacteria caused by growth and aging. Following the spectra analysis, a SVM (support vector machine) classification based on growth phases is presented. In sum this longitudinal study performed with a compact and low-cost Raman setup is a proof of principle for routine analysis of bacteria, in a real-time and non-destructive way. Real-time Raman studies on metabolism and viability of bacteria paves the way for future antibiotic susceptibility testing.

8939-5, Session 1

Cell identification using Raman spectroscopy in combination with optical trapping and microfluidics

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Raman activated cell sorting (RACS) offers prospects to complement the widely applied fluorescence activated cell sorting. RACS can be realized by combination with optical traps and microfluidic devices. The progress of RACS is reported for a cellular model system that can be found in peripheral blood of tumor patients. Lymphocytes and erythrocytes were extracted from blood samples. Breast carcinoma derived tumor cells (MCF-7, BT-20) and acute myeloid leukemia cells (OCI-AML3) were grown in cell cultures. First, Raman images were collected from dried cells on calcium fluoride slides. Support vector machines (SVM) classified 99.7% of the spectra to the correct cell type. Second, a 785 nm laser was used for optical trapping of single cells in aqueous buffer and for excitation of the Raman spectrum. SVM distinguished 1210 spectra of tumor and normal cells with a sensitivity of >99.7% and a specificity of >99.5%. Third, a microfluidic glass chip was designed to inject single cells, modify the flow speed, accommodate fibers of an optical trap and sort single cells after Raman based identification with 514 nm for excitation. Forth, the microfluidic chip was fabricated by quartz which can improved cell identification results with 785 nm excitation. Finally, a Raman-on-chip approach was developed that integrates fibers for trapping, Raman excitation and signal detection in a single compact unit.

8939-6, Session 1

Label-free haemogram using wavelength modulated Raman spectroscopy for identifying immune-cell subset

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Leucocytes in the blood of mammals form a powerful protective system against a wide range of dangerous pathogens. There are several types of immune cells that has specific role in the whole immune system. The number and type of immune cells alter in the disease state and identifying the type of immune cell provides information about a person's state of health. There are several immune cell subsets that are essentially morphologically identical and require external labeling to discriminate between. Here we demonstrate for the first time the feasibility of using Wavelength Modulated Raman Spectroscopy (WMRS) with suitable machine learning algorithms as a label-free method to distinguish between different closely lying immune cell subset. Principal Component Analysis (PCA) was performed on WMRS data from single cells, obtained using confocal Raman microscopy for feature reduction, followed by Support Vector Machine (SVM) for binary discrimination of various cell subset, which yielded an accuracy >85%. The method was successful in discriminating between untouched and unfixed purified populations of CD4+CD3+ and CD8+CD3+ T lymphocyte subsets, and CD56+CD3- natural killer cells with a high degree of specificity. It was also proved sensitive enough to identify unique Raman signatures that allow clear discrimination between dendritic cell subsets, comprising CD303+CD45+ plasmacytoid and CD1c+CD141+ myeloid dendritic cells. The results of this study clearly show that WMRS is highly sensitive and can distinguish between cell types that are morphologically and chemically closely related.

8939-7, Session 2

Multimodal nano-bioprobes for imaging EGFR on single human cancer cells

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Multimodal nanoparticles, by integrating different diagnostic capabilities into one single nanostucture, can overcome the limitations of current medical assays in sensitivity and resolution and therefore improve their clinical value. Here we report a novel nanomaterial composed of gadolinium oxide (Gd2O3)-doped silica nanoparticles and gold nanoparticles (Gd-Au NPs) that can be used as a multimodal bioprobes for Surface-enhanced Raman spectroscopy (SERS), fluorescence and confocal reflectance imaging. In this bioprobe, the gold nanoparticles serve as hot spots and generate SERS signals.

Gd-Au NPs were conjugated with antibody to epidermal growth factor receptor (EGFR), a biomarker molecule for most cancers, to localize the spatial distribution of EGFR on cell surface. The characteristic SERS peaks from Raman reporter molecule, 4-mercaptobenzoic acid (MBA), were observed at 1077 cm-1 and 1588 cm-1. By comparing the SERS intensities at 1077 cm-1, we have shown that anti-EGFR-conjugated Gd-Au NPs can generate the highest SERS signals, due to the specific antibody-antigen recognition on cell surface. Moreover, we compared the EGFR expression level and mapped the cellular EGFR localization on the model cancer cell lines, human epidermoid carcinoma cell (A431), human lung adenocarcinoma cell (A549) and human nasopharyngeal carcinoma cell (S18). The SERS mapping along with fluorescence and confocal reflectance images together have shown the EGFR distribution at single-cell level. Thus, our study shows the potential of Gd-Au NP as a multimodal imaging agent to map cancer biomarkers at molecular and sub-cellular level.

8939-8, Session 2

New SERS: scattering enhanced Raman scattering

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Raman spectroscopy is a powerful technique that can be used to obtain detailed chemical information about a system without the need for chemical markers. It has been widely used for a variety of applications such as cancer diagnosis and material characterization. However, Raman scattering is a highly inefficient process, where one in 10^11 scattered photons carry the needed information. Several methods have been developed to enhance this inherently weak effect, including surface

enhanced Raman scattering and coherent anti-Stokes Raman scattering. These techniques suffer from drawbacks limiting their commercial use, such as the need for spatial localization of target molecules to a 'hot spot', or the need for complex laser systems.

Here, we present a simple instrument to enhance spontaneous Raman scattering using elastic light scattering. Elastic scattering is used to substantially increase the interaction volume [1]. Provided that the scattering medium exhibits very low absorption in the spectral range of interest, a large enhancement factor can be attained in a simple and inexpensive setting. In our experiments, we demonstrate an enhancement of 10^6 in Raman signal intensity. The proposed novel device is equally applicable for analyzing solids, liquids, and gases.

In our presentation, we will explain the theoretical basis for such signal enhancement, describe the experimental setup, and illustrate the great potential of this device for detecting low concentrations of contaminates. Finally, we will discuss the ultimate limits of detection sensitivity and evaluate the device's performance for near real-time analysis in applications ranging from cancer detection to water purity analysis.

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8939-9, Session 2

SERS-barcoded colloidal gold NP assemblies as imaging agents for use in biodiagnostics

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There is a growing need for new biodiagnostics that combine high throughput with enhanced spatial resolution and sensitivity. Gold nanoparticle (NP) assemblies with sub-10 nm particle spacing have the benefits of improving detection sensitivity via Surface enhanced Raman scattering (SERS) and being of potential use in biomedicine due to their colloidal stability. A promising and versatile approach to form solution-stable NP assemblies involves the use of multi-branched molecular linkers which allows tailoring of the assembly size, "hot-spot" density and interparticle distance.1 We have shown that functional multi-branched or hyperbranched polymer (HBP) architectures with multiple anchoring end-groups can be successfully employed as a linker to assemble gold NPs into NP assemblies.2 These NP assemblies with diameters of 50-120 nm are stable in solution and perform better as SERS substrates compared with single gold NPs, due to an increased "hot-spot" density. Thus, HBP mediated gold NP assemblies are potential candidates for use as biomedical imaging agents. We witnessed that the "hot-spot" density and in-turn the SERS enhancement is a function of the HBP concentration and the polymer architecture. New deep Raman techniques like Spatially Offset Raman Spectroscopy (SORS) have emerged that allow detection from beneath diffusely scattering opaque materials, including biological media such as animal tissue.3 We have been able to demonstrate that the gold NP assemblies could be detected from within both proteinaceous and high lipid containing animal tissue with a backscattered SORS geometry. We have also studied our NP assemblies in comparison to those available in the market.

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8939-10, Session 2

Application of online Fourier Transform CARS

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Coherent anti-Stokes Raman scattering (CARS) microscopy is an important technique for biological imaging due to its nonlinear nature in signal generation. Specific molecular vibrations can be accessed without the need of fluorescent labeling avoiding any photo bleaching or toxicities of the sample. Conventional implementations of CARS microscopy require two narrow band laser sources to match one vibrational frequency of a known molecule. With the combination of a broad- and narrowband pulse a whole spectrum of vibrational modes can be accessed at once (Multiplex CARS). Both approaches lack in efficient removing of the non-resonant background and spectral resolution depending on the probe pulse. This limitation restricts imaging in the fingerprint region due to the large amount of crowded molecular bands with various bandwidths. A method to efficiently suppress the non-resonant background and achieve high spectral resolution while using a single laser source is presented here. The vibrational excitation of the sample is performed by impulsive stimulated Raman scattering and probed with a second time delayed probe pulse to time resolve the CARS signal. The time resolved CARS signal and the background free CARS spectrum can be acquired over the entire fingerprint region simultaneously and offering great potential for fast histo- and cytopathology. We demonstrate the feasibility of this modality in phantoms (polystyrene beads, PMMA) and biological samples (human bone tissue).

8939-12, Session 2

Hyperspectral Raman imaging (HSRI) by active-illumination for molecular imaging

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Raman spectroscopy can provide molecular information via inelastic light scattering without physical contact. Coupled with microscopic imaging, Raman imaging is a powerful technique for material analysis, for example, stress and temperature measurement in silicon and compositional analysis of polymer microparticles. However, due to the small Raman cross-section, the data acquisition time is significantly longer than other optical modalities. The traditional design of conventional charge coupled detector (CCD) readout electronics introduces additional latency, resulting in slow Raman imaging throughput.

Recently, we have developed a novel parallel Raman imaging scheme for simultaneously collecting Raman spectra from multiple points [1]. This scheme is realized by multiple-point laser active-illumination using a spatial light modulator (SLM) coupled with wide-field Raman image collection. We have demonstrated the performance of this scheme using uniform samples, fixed polymer microparticles and trapped polymer microparticles with mixed molecular composition within a ~ 100 x 100 ?m2 field of view This scheme enables the acquisition of Raman spectra from as many as 80 laser spots (equivalent to ~200 diffraction limited imaging pixels) simultaneously using a single illumination pattern and detector recording frame without scanning.

In this paper, we will discuss strategies for extending this technique to continuous image acquisition via multiple pre-designed illumination patterns, where streamlined pattern illumination and synchronized Raman data collection is key. We will also present results on snapshot image guided Raman/SERS acquisition, in which a snapshot brightfield image is first taken and analyzed to extract features of interest, and a series of illumination patterns are subsequently generated for Raman/SERS acquisition. As a result, the throughput of this scheme can be orders of magnitude faster than commercial systems.

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8939-13, Session 2

A novel method for single bacteria identification by Raman spectroscopy

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In this paper we present results on single bacteria rapid identification obtained with a low-cost and compact Raman spectrometer. At present, we demonstrate that a 1 minute procedure, including the localization of single bacterium, is sufficient to acquire comprehensive Raman spectrum in the range of 600 to 3300 cm-1. Localization and detection of single bacteria is performed by means of lensfree imaging over a large field of view of 24mm2. An excitation source of 532nm and 30mW illuminates single bacteria to collect Raman signal into a Tornado Spectral Systems prototype spectrometer (HTVS technology). The acquisition time to record a single bacterium spectrum is as low as 10s owing to the high light throughput of this spectrometer. The spectra processing features different steps for cosmic spikes removal, background subtraction, and gain normalization to correct the residual inducted fluorescence and substrate fluctuations. This allows obtaining a fine chemical fingerprint analysis. We have recorded a total of 1500 spectra over 10 bacterial species (E.coli, Bacillus species, S.epidermis, M.luteus, S.marcescens). The analysis of this database results in a high classification score of about 90%. Hence we can conclude that our setup enables automatic recognition of bacteria species among 10 different species. The speed and the sensitivity (<30 minutes for localization and spectra collection of 30 single bacteria) of our Raman spectrometer pave the way for high-throughput and non-destructive real-time bacteria identification assays. This compact and low-cost technology can benefit biomedical, clinical diagnostic and environmental applications.

8939-14, Session 3

Classification of oral cancers using Raman spectroscopy of serum

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Oral cancers are the sixth most common malignancy worldwide, with low 5-year disease free survival rates, attributable to late detection due to lack of reliable screening modalities. Our in vivo Raman spectroscopy studies have demonstrated classification of normal and tumor as well as cancer field effects (CFE), the earliest events in oral cancers. In view of limitations such as requirement of on-site instrumentation and stringent experimental conditions of this approach, feasibility of classification of normal and cancer using serum was explored using 532 nm excitation. In this study, strong resonance features of ?-carotenes, present differentially in normal and pathological conditions, were observed. In the present study, Raman spectra of sera of 36 buccal mucosa, 33 tongue cancers and 17 healthy subjects were recorded using Raman microprobe coupled with 40X objective using 785 nm excitation, a known source of excitation for biomedical applications. To eliminate heterogeneity, average of 3 spectra recorded from each sample was subjected to PC-LDA followed by leave-one-out-cross-validation. Findings indicate average classification efficiency of ~70% for normal and cancer. Buccal mucosa and tongue cancer serum could also be classified with an efficiency of ~68%. Of the two cancers, buccal mucosa cancer and normal could be classified with a higher efficiency. Findings of the study are quite comparable to that of our earlier study, which suggest that there exist significant differences, other than ?-carotenes, between normal and cancerous samples which can be exploited for the classification. Prospectively, extensive validation studies will be undertaken to confirm the findings.

8939-15, Session 3

Raman spectroscopy for the assessment of acute myeloid leukemia: a proof of concept study

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Acute myeloid leukemia (AML) is the most common type of leukemia in adults and it is associated with the lowest survival rate of all leukemia types (Deschler et al. 2006). AML is classified in several subtypes, different in morphologic/immunophenotypic characteristics, cytogenetic and genetic abnormalities, prognosis and clinical features. Consequently, the correct and rapid assessment of AML is crucial for the definition of correct prognosis and successful treatments (Döhner et al. 2010).

The morphological evaluation of blood is the first essential step of the diagnosis of AML, followed by immunophenotypical, cytogenetic and molecular evaluations. Nowadays, the morphological evaluation is manually assessed on stained smears and is therefore subjective, error-prone, time-consuming and requires highly skilled hematologists.

For this reason is necessary to introduce new methods suitable for automated high-throughput screening for a more reproducible and objective first-assessment of AML subtypes.

Raman spectroscopy is a promising label-free method for the characterization and distinction of different cells on the basis of their chemical composition and their difference in the spatial molecular distribution at subcellular level (Wachsmann-Hogiu et al, 2009).

Here we present high-resolution Raman mapping of cells from patients with different AML subtypes (e.g. AML without differentiation, acute promyelocytic leukemia and acute erythroid leukemia) classified by traditional morphological, immunophenotypical and genetic evaluations. Cells from patients affected by different AML subtypes were efficiently distinguished on the basis of the detection of typical features related to the lineage and the stage of differentiation of those cells.

These data are promising for the development and use of Raman spectroscopy as tool for the assessment of AML subtypes based on an efficient, objective and potentially automatable label-free method.

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8939-16, Session 3

Vibrational imaging unveils the essential role of cholesterol accumulation in cancer proliferation

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Altered lipid metabolism is increasingly recognized as a signature of cancer cells. As an essential aspect of lipid metabolism, storage of esterified lipids in lipid droplets remains underappreciated, even though lipid accumulation has been described in multiple cancers. Particularly, because composition of lipid droplets at the single cell level cannot be readily assessed with traditional methods, the exact role of lipid accumulation in cancer remains elusive. Enabled by label-free coherent Raman scattering microscopy, we performed the first quantitative analysis of lipogenesis at single cell level in human patient cancerous tissues. Our study revealed an unexpected, aberrant accumulation of esterified cholesterol in lipid droplets of high-grade prostate cancer and metastases. Such cholesteryl ester accumulation was caused by loss of tumor suppressor PTEN and consequent activation of PI3K/AKT pathway that bypasses androgen receptor. Pharmacological depletion of cholesteryl ester storage restored intracellular cholesterol homeostasis and significantly impaired cancer aggressiveness with negligible toxicity. These findings reveal a novel target for diagnosis and treatment of aggressive human prostate cancer.

8939-17, Session 3

The use of confocal Raman microscopy to study the effects of phenothiazine derivatives in human colon cancer cells

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Confocal Raman microscopy, a non-invasive, label free and high spatial resolution imaging technique is used to study the effects of methylene blue and toluidine blue in living HCT-116 cells – a human colon cancer cell line (Fig.1A,B). The images of the phenothiazine compound localized in cells are obtained from the Raman spectra treated by K-mean cluster analysis1 method (Fig.1C,D). Under similar treatment conditions, toluidine blue preferentially binds to the cells and is localized in the cytoplasm. Tracing cell apoptosis, we observe a decrease in the intensity of cytochrome c after treatment with phenothiazines. Further cell damage is evident in the loss of cell adhesion. The p53 deleted HCT-116 cells are more resistant to the treatment suggesting a role for p53 induced apoptosis2.

8939-18, Session 3

Micro-Raman spectroscopy studies of changes in lipid composition in breast and prostate cancer cells treated with MPA and R1881 hormones

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Increasing interest in the role of lipids in cancer cell proliferation or resistance to drug therapies has motivated the need to develop better tools for cellular lipid analysis. Quantification of lipids in cells is typically done by destructive chromatography protocols that do not provide spatial information on lipid distribution and prevent dynamic live cell studies. Methods that allow the analysis of lipid content in live cells is therefore of great importance for research. Using Raman micro-spectroscopy we investigated whether the female hormone medroxyprogesterone acetate (MPA) and the synthetic androgen R1881 affect the lipid expression in breast (T47D) and prostate (LNCaP) cancer cells. Differences were noted in the spectral regions at 830-1800 cm-1 and 2800-3000 cm-1 when comparing different drug treatments. Significant changes were noticed for saturated (1063 - 1125 cm-1, 1295 cm-1 and 1439 cm-1), unsaturated (1262 cm-1 and 1656 cm-1, and 1720 – 1748 cm-1) chemical bonds, suggesting that the composition of the lipid droplets was changed by the hormone treatments. Also, significant differences were observed in the high frequency regions of lipids and proteins at 2851 cm-1 and around 2890 cm-1. Principal component analysis with Linear Discriminant Analysis (PCA-LDA) of the Raman spectra was able to differentiate between cancer cells that were treated with MPA, R1881 or vehicle ($P < 0.05$). Future work includes analysis to determine exact lipid composition and concentrations as well as development of clinical techniques to characterize differences in patient tumor lipid profiles to determine response to drug treatment and prognosis.

8939-19, Session 3

Investigating the biochemical progression of liver disease through fibrosis, cirrhosis, dysplasia, and hepatocellular carcinoma using Fourier transform infrared spectroscopic imaging

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Hepatocellular carcinoma (HCC) is the most common form of primary hepatic carcinoma. HCC ranks the fourth most prevalent malignant tumor and the third leading cause of cancer related death in the world. Hepatocellular carcinoma develops in the context of chronic liver disease and its evolution is characterized by progression through intermediate stages to advanced disease and possibly even death. The primary sequence of hepatocarcinogenesis includes the development of cirrhosis, followed by dysplasia, and hepatocellular carcinoma. We addressed the utility of Fourier Transform Infrared (FT-IR) spectroscopic imaging, both as a diagnostic tool of the different stages of the disease and to gain insight into the biochemical process associated with disease progression. Tissue microarrays were obtained from the University of Illinois at Chicago tissue bank consisting of liver explants from 45 transplant patients. Three tissue core biopsies were obtained from each explant targeting regions of fibrosis, dysplasia and hepatocellular carcinoma. We obtained FT-IR images of these tissues using a modified FT-IR system with high definition capabilities. Firstly, the major cell types of the liver tissue were accurately segmented using a supervised spectral classifier. Secondly, a classifier was built to discriminate the different grades of liver disease. Lastly, spectra were extracted from the different liver cell types to identify the biochemical changes observed as the disease progressed to hepatocellular carcinoma. With the emerging advances in FT-IR instrumentation and computation there is a strong drive to develop this technology as a powerful adjunct to current histopathology approaches to improve disease diagnosis and prognosis.

8939-20, Session 4

A pilot study on Raman mapping of normal and cancerous oral tissues

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Raman spectroscopy has shown significant potential in the characterization and clinical diagnosis of normal and cancerous tissues. Many have reported that the fingerprint region provides a wealth of information which can be exploited to identify and characterise the changes that occur during the transformation of normal into cancer cells. However, not much study on the comparison of Raman mapping of normal and cancerous oral tissues has been done. In this regard, a pilot study was carried out on mapping of five normal and three oral cancer tissues using MicroRaman Confocal system. Considerable differences between the noncancerous and cancerous oral tissues were found due to the variation in the vibrational characteristics of carotenoids, fatty acids and proteins. Raman spectra collected from the cancer tissues consistently showed lesser collagen content when compared with normal tissue spectra obtained through this mapping study. Cluster analysis was performed for each image which resulted in groups of similar spectra. Pseudocolour Raman maps of the tissues were constructed by assigning different colours for different clusters. Following Raman mapping, the tissue sections were stained for histopathological analysis, enabling identification of the histological origin of the Raman signature and assignment of the Raman spectral clusters to different regions of the mapped tissue. The results would be discussed.

8939-21, Session 4

Combined information from Raman spectroscopy and optical coherence tomography for enhanced diagnostic accuracy in tissue discrimination

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Optical spectroscopy and imaging methods have proved to have potential to discriminate between normal and abnormal tissue types through minimally invasive procedures. Raman spectroscopy and Optical Coherence Tomography (OCT) provides chemical and morphological information of tissues respectively that are complementary to each other. When used individually they might not be able to obtain high enough sensitivity and specificity that is clinically relevant. In this study we combined Raman spectroscopy information with information obtained from OCT to enhance the sensitivity and specificity in discriminating between Colonic Adenocarcinoma from Normal Colon. OCT being an imaging technique, the information from this technique is conventionally analyzed qualitatively. To combine with Raman spectroscopy information, it was essential to quantify the morphological information obtained from OCT. Texture analysis was used to extract information from OCT images, which in-turn was combined with the information obtained from Raman spectroscopy. The sensitivity and specificity of the classifier was estimated using leave one out cross validation method where support vector machine was used for binary classification of the tissues. The sensitivity obtained using Raman spectroscopy and OCT individually was 89% and 78% respectively and the specificity was 77% and 74% respectively. Combining the information derived using the two techniques increased both sensitivity and specificity to 94% demonstrating that combining complementary optical information enhances diagnostic accuracy. These results demonstrate that a multimodal approach using Raman-OCT would be able to enhance the diagnostic accuracy for identifying normal and cancerous tissue types.

8939-22, Session 4

Real-time depth-resolved fiber optic Raman endoscopy for in vivo diagnosis of gastric precancer

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Raman spectroscopy is a vibrational analytic technique sensitive to the changes in biomolecular composition and conformations occurring in tissue. With our most recent development of on-line near-infrared (NIR) Raman endoscopy integrated with a depth-resolving fiber-optic Raman probe for selective interrogation of the epithelium, in vivo Raman tissue diagnosis (optical biopsy) during clinical gastrointestinal endoscopy has been realized under multimodal wide-field imaging (i.e., white- light reflectance (WLR), narrow-band imaging (NBI), autofluorescence imaging (AFI)) guidance.

A selection of 450 patients who previously underwent Raman endoscopy (n=1900 spectra) was used to render diagnostic models for identifying gastric dysplasia based on probabilistic partial least squares - discriminant analysis (PLS-DA). The on-line Raman endoscopy technique was tested prospectively on (n=5) patients for real-time in vivo epithelium tissue diagnosis.

High quality in vivo Raman spectra can be acquired and classified in real-time within 0.5 sec during clinical endoscopic examinations. Substantial differences in Raman spectra between normal and dysplastic tissue are observed mainly associated with upregulated protein synthesis and elevated DNA content reflecting the onset of gastric carcinogenesis. The fiber-optic Raman endoscopic technique developed could prospectively identify gastric dysplasia in real-time with a sensitivity: 81.3% (61/75) and specificity 88.3% (188/213) on spectrum basis. On lesion basis, all dysplastic lesions were identified.

This study demonstrates for the first time the prospective real-time depth-resolved Raman endoscopy for in vivo diagnosis of precancer in the gastric at the molecular level.

8939-23, Session 4

Performing independent validation of Raman spectroscopy for cervical precancer detection in vivo

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No Abstract Available

8939-24, Session 5

The discrimination of fish egg quality and viability by using Raman spectroscopy

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Sexual reproductive body can be produced from a fertilized ovum. Once the ovum is fertilized with sperm, it runs through the cell division, differentiates to all kinds of cells, and goes to make a complete body. However, not all of them are viable and some of them stop to

ontogenesis showing the developmental abnormality. The ovum quality is now assessed by morphology and cleavage rate of embryo. In order to bring out the better outcome for aquaculture or in vitro fertilization (IVF) treatment, we expect to be able to assess and choose the viable embryo by investigating the embryonic quality based on the scientific data as molecular composition information.

In our research, we measure the Raman spectra from fish egg; Japanese Medaka (Oryzias Latipes) using a microscope Raman system which consists of a 785nm diode laser (Toptica Photonics, Germany), Raman spectrometer (F=4.2, focal length 320nm, 750nm blazed 600 l/mm grating; Photon Design Co. Ltd. Japan), and charge coupled device detector (CCD; DU420-BRDD, ANDOR Technology Co. Ltd., Northern Ireland) with microscope (Mitutoyo M11002805A, 20?/0.40, f=200). After the measurement, these Raman data are checked up with the information about the eggs can survive or not, and we examine what factors are important in egg components to distinguish between “good quality” and “not good quality”. We present the results of assessment of egg quality, and investigate whether Raman spectroscopy can be used to a discriminate of egg quality.

8939-25, Session 5

Monitoring the influence of antibiotic exposure using Raman spectroscopy

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Antibiotics stop bacterial growth and cure infection through two main mechanisms which are either bactericidal or bacteriostatic. Bactericidal antibiotics kill the bacteria, and bacteriostatic antibiotic prevent the growth of bacteria. . One of many factors to predict a favorable clinical outcome of the potential action of antimicrobial may be provided using in vitro bactericidal/bacteriostatic data (e.g. minimum inhibitory concentrations – MICs). Consequently, MICs are used in clinical situations mainly to confirm resistance, and to determine the in vitro activities of new antimicrobials.

Here we report on combination of the data obtained from MICs with information of microoragnisms „fingerprint“ provided by Raman spectroscopy. The most prominent features observed in the Raman spectra of bacterial colonies can be broadly categorized into four groups – proteins, DNA/RNA, sugars, and lipids. In our feasibility study we could follow mechanisms of the bacteriostatic versus bactericidal action simply by monitoring Raman bands corresponding to DNA translating the changes introduced by selected antibiotics. The Raman spectra of Staphylococcus epidermidis treated with bacteriostatic agent show little effect on DNA which is in contrast with the action of bactericidal agent where Raman spectra show decreased in DNA signal strength suggesting DNA fragmentation.

8939-26, Session 5

Raman spectroscopic estimation of myocardial infarction

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Myocardial infarction (MI) following ischemia is a major cause of mortality worldwide. The determination of treatment of MI is based on myocardial viability (MV), which is the potential to recover functions of myocardium. In general, magnetic resonance imaging and myocardial scintigraphy are used for the estimation of MV before cardiac surgery. However, MV should be estimated during the surgery for the precise treatment of MI. An intraoperative estimation method of MV is therefore deeply desired for better outcome of cardiac surgery. In our previous repot, we have demonstrated an estimation method of the MV by visualizing intact and old infarcted myocardial regions with Raman spectroscopy (RS). In this study, we provided a proof-of-principle demonstration of an estimation technique of MV in fresh MI by using RS. Since the MV depends on the tissue species of myocardium, we sought to classify the tissue species of normal hearts and those with fresh MI by using RS analysis. MI was created by complete ligation of the left descending coronary artery of Wistar rats. Firstly, we obtained Raman spectra of normal hearts and those with fresh MI of the Wistar rats, and extracted specific spectral features for them without any preprocessing, neither fixation nor staining. Secondly, by applying multivariate spectral analysis, we successfully classified tissue species of the normal and the infarcted hearts. These results suggest the potential of the RS observation for noninvasive and label-free estimation of MV, and we expect that this method could become a key technique for cardiac surgery.

8939-27, Session 5

Endoscopy-coupled Raman spectroscopy for in vivo discrimination of inflammatory bowel disease

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Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn’s colitis (CC), affects nearly 2 million Americans, and the incidence is increasing worldwide. It has been established that UC and CC are distinct forms of IBD and require different medical care. Currently, the distinction made between UC and CC is based upon inexact clinical, radiological, endoscopic, and pathologic features. A diagnosis of indeterminate colitis occurs in up to 15% of patients when UC and CC features overlap and cannot be differentiated. In these patients, diagnosis relies on long term follow up based on success or failure of existing treatment and recurrence of the disease. Thus, there is need for a tool that can improve the sensitivity and specificity for fast, accurate and automated diagnosis of IBD. Here we present colonoscopy-coupled fiber optic probe-based Raman spectroscopy as a novel diagnostic tool for IBD. This in vivo study of patients with existing IBD diagnoses of UC (N=13) and CC (N=21) aims to characterize spectral signatures of UC and CC. Samples are correlated with tissue pathology markers, and endoscopic evaluation. Optimal collection parameters for detection have been identified based upon instrument design. The collected spectra are processed and analyzed using multivariate statistical techniques to identify spectral markers and discriminate UC and CC. Development of spectral markers to discriminate disease type is a necessary first step in the development of real-time, accurate and automated in vivo detection of IBD during colonoscopy procedures.

8939-28, Session 5

Mid-infrared laser spectroscopy in vivo

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The biomedical application of mid-infrared spectroscopy is often hampered by the high absorption of mid-infrared radiation caused by water. In our research we strongly mitigate the impact of this absorption with the goal to continuously monitor of the concentration of glucose in tissue. A quantum cascade laser and a mid-infrared fiber are used for the reagent-free continuous glucose monitoring, whereby a narrow gap in the fiber serves as cuvette of low transmission length. Biocompatibility testing is performed prior to applying the fiber sensor to the subcutaneous tissue.

8939-29, Session 5

Fourier transform infrared spectroscopic imaging identifies early biochemical markers of tissue damage

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Fourier Transform Infrared (FT-IR) spectroscopic imaging can allow for the rapid imaging of tissue biochemistry in a label-free and non-perturbing fashion. With the rapid adoption of new minimally invasive surgery (MIS) technologies over the last 20 years, adequate skill to safely and effectively use these technologies may not be achieved and risk of undue physical pressure being placed on tissues is a concern. Previous work has demonstrated that a number of histological stains can detect tissue damage, however, this process requires the initiation and progression of a signaling cascade that results in the epitope of interest being expressed. We proposed to identify the early biochemical markers associated with physical tissue damage from applied forces, thus not requiring transcriptional and translational protein synthesis as traditional immunohistochemistry does. To demonstrate that FT-IR can measure biochemical changes in tissues that have undergone physical force, we took ex vivo lambs livers that had been freshly excised and applied varying levels of physical pressure (0N to 300N). Tissues were then formalin-fixed, paraffin-embedded, and sectioned on to glass for staining for cell types, cell death and blood clotting and on to an IR slide for FT-IR imaging. Regions of interest containing hepatocytes were identified and average FT-IR spectra were extracted from the damaged and undamaged livers. FT-IR spectra showed clear biochemical changes associated with tissue damage even at low pressures. In addition, chemical changes could be observed preceding histological changes using conventional staining approaches.

8939-11, Session 6

Understanding the TERS effect with on-line tunneling and force feedback using multiprobe AFM/NSOM with Raman integration

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Tip enhanced Raman scattering (TERS) has evolved in several directions over the past years. The data from this variety of methodologies has now accumulated to the point that there is a reasonable possibility of evolving an understanding of the underlying cause of the resulting effects that could be the origin of the various TERS enhancement processes. The objective of this presentation is to use the results thus far with atomic force microscopy (AFM) probes with noble metal coating, etching, transparent gold nanoparticles with and without a second nanoparticle [Wang and Schultz, ANALYST 138, 3150 (2013)] and tunneling feedback probes [R. Zhang et. al., NATURE 4 9 8, 8 2 (2 0 1 3)]. We attempt at understanding this complex of results with multiprobe techniques of two gold nanoparticles with controlled separation. This complex quantum system enters, in the near-field, into a regime of extreme non-locality. This produces a highly confined and broadband plasmon field with all k vectors for effective excitation. Normal force tuning fork feedback with exposed tip probes provides an excellent means to investigate these effects with TERS probes that we have shown can circumvent the vexing problem of jump to contact and permit on-line switching between tunneling and AFM feedback modes of operation

8939-30, Session 6

Multimodal fiber probe spectroscopy for tissue diagnostics applications: a combined Raman-fluorescence approach

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Two different optical fiber probes for combined Raman and fluorescence spectroscopic measurements were designed, developed and used for tissue diagnostics. Two visible laser diodes were used for fluorescence spectroscopy, whereas a laser diode emitting in the NIR was used for Raman spectroscopy. The two probes were based on fiber bundles with a central multimode optical fiber, used for delivering light to the tissue, and 24 surrounding optical fibers for signal collection. Both fluorescence and Raman spectra were acquired using the same detection unit, based on a cooled CCD camera, connected to a spectrograph. The two probes were successfully employed for diagnosing melanocytic lesions in a good agreement with common routine histology. The obtained results demonstrated that the multimodal approach is crucial for improving diagnostic capabilities. Further investigations were performed on colon and brain tissue samples in order to have a benchmark for diagnosing a broader range of tissue lesions and malignancies. The system presented here can improve diagnostic capabilities on a broad range of tissues and has the potential of being used for endoscopic inspections in the near future.

8939-31, Session 6

Quantitative fiber optic Raman spectroscopy for tissue Raman measurements

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Molecular profiling of tissue using near-infrared (NIR) Raman spectroscopy has shown great promise for in vivo detection and prognostication of cancer. The Raman spectra measured from the tissue generally contain fundamental information about the absolute biomolecular concentrations in the tissue and its changes associated with disease transformation. However, producing analogues tissue Raman spectra present a great technical challenge to the biomedical community in pushing Raman spectroscopy for in vivo real-time diagnosis of cancers in the clinic. Normalization is the most widespread method to make the diagnosis based on the spectral shape information, while disregarding the quantitative (intensity) information. In this study, we propose a calibration method to ensure the reproducible tissue Raman measurements and validated with the acquired in vivo palm spectra using different fiber-optic probes. A rapid Raman spectroscopy system coupled with a ball-lens fiber-optic Raman probe was utilized for tissue Raman

measurements. The investigational results showed that the variations between the spectra measured with two probes are almost negligible, facilitating the quantitative analysis of tissue Raman measurements in vivo.

8939-32, Session 6

A low background Raman probe for optical biopsy of brain tissue

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Removal of brain tumours is a delicate process, where precision is required to remove all of the cancerous tissue without damaging healthy brain tissue. The accuracy of this process, currently performed by eye, could be greatly improved by use of 'optical biopsies' using Raman spectroscopy.

A miniature Raman probe for performing optical biopsies of human brain tissue is presented. The probe allows sampling inside a conventional stereotactic brain biopsy system: a needle of length 200mm and inner diameter of 1.8mm.

The probe achieves a very low auto-fluorescent background whilst maintaining good collection of Raman signal by employing a miniature stand-off Raman design. To illustrate this, the probe is compared with a conventional Raman probe that uses a pair of optical fibres for collection. The miniature stand-off Raman probe is shown to have equal Raman signal collection ability, but the significant fluorescence caused by silica fibres in a conventional Raman needle probe is reduced by a factor of two for Raman shifts under 500 cm-1, and by 30% at 600-700 cm-1. In addition, this design contains only medically approved materials at the distal end.

Once ethical approval has been obtained, the probe will be used to attempt to discriminate between healthy and cancerous brain tissue.

8939-33, Session 6

Clinical Raman measurements under special ambient lighting illumination

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Raman spectroscopy is a non-invasive optical technique that is widely used in the investigation of medical diagnosis. One challenge facing Raman spectroscopy is that the signal is exceedingly weak, requiring a relatively long integration time with little background light interference. Using skin Raman measurements as an example, it is inconvenient for the patient and the operator to be kept in darkness, where the computer monitors may also need to be turned off, difficult for the operator to locate the target site. Therefore, it is highly desirable to be able to carry out Raman measurement with ambient lighting that substantially reduces these problems but has little affect on the Raman signal. We proposed a method for Raman measurement with shaped ambient lighting and Raman collection optics. Results are presented for skin Raman measurements with 785 nm excitation. The ambient room illumination was replaced with either a broadband white light LED lamp spectrally shaped with a broad band-pass filter between 350 and 700 nm, or with a white LED lamp consisting of red, green, blue composite LEDs without near IR components. A special long-pass filter was placed in the Raman collection arm so that the ambient light between 350 and 700 nm was rejected. The CRT and LCD monitor were also replaced with LED monitor. It was found that reliable skin Raman spectra could be acquired with these special ambient illumination arrangements. The LED monitor can be kept on during the measurement to display the results. This method is also applicable to other organ sites and laboratory/industrial settings

8939-34, Session 6

Application of the shifted excitation Raman difference spectroscopy (SERDS) to the analysis of trace amounts of methanol in red wines

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Shifted Excitation Raman Difference Spectroscopy (SERDS) has proven an effective method for performing Raman analysis of fluorescent samples. This technique allows achieving excellent signal to noise performance with shorter excitation wavelengths, thus taking full advantage of the superior signal strength afforded by shorter excitation wavelengths and the superior performance, also combined with lower cost, delivered by silicon CCDs. The technique is enabled by use of two closely space fixed-wavelength laser diode sources stabilized with the Volume Bragg gratings (VBGs). We present a detailed quantitative analysis of the advantages of this technique over the conventional methods employed in Raman analysis, such as baseline fitting and numerical differentiation. We also show a side by side comparison of SERDS with Raman analysis performed using longer laser wavelengths (e.g. 1064 nm) on the same samples. We show that SERDS technique delivers superior signal to noise ratio and better detection limits in most situations, even when a longer excitation wavelength is employed for the purpose of elimination of the fluorescence. SERDS technique is then applied to the quantitative analysis of the presence of trace amounts of methanol in red wines, which is an important task in quality control operations within wine industry and is currently difficult to perform in the field. So far conventional Raman spectroscopy analysis of red wines has been impractical due to the high degree of fluorescence found in red wines.

8939-35, Session 6

Rapid hyperspectral imaging in the mid-infrared

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Despite the successes of mid-infrared hyperspectral imaging in a research environment, the migration of technology into the day-to-day clinical application is hardly progressing. Clinical acceptance may be improved if the spectroscopy were faster and the overall microscope were less expensive. Here we present first results of a fast, multi-scale mid-infrared microscopy setup which allows for the investigation of 16x12 mm? and 4x3mm? fields of view with a resolution of 28µm and 14 µm, respectively, in less than 20s per hyperspectral image. Two tunable quantum cascade lasers with tuning ranges of up to 300 cm-1 serve as light sources. Imaging is accomplished using a microbolometer array.

8939-36, Session 6

Flexible highly sensitive protected ATR FTIR fiber sensor from nanostructured silver halides for spectroscopic (500-4000 cm-1) characterization of soft tissue in vivo

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We present new stable mid-IR fiber ATR sensor with record transmission properties. Multimode core-clad fiber was extruded of silver halide and has smooth absorption spectrum of losses from 0.1 dB/m @ 1000 cm-1 to 1.5 dB/m @ 4000 cm-1. Sensor head was made of half sphere diameter 2 mm with impregnation of nano-diamonds and Ag2S, which enhance surface evanescent absorption by mid-IR plasmons and improve durability. Maximum diameter of protected sensor is less than 2.5 mm. This sensor with FTIR spectrometer with standard detector options gives low noise spectrum. We studied effects of different visible light exposure on humans skin during clinic dermatological physiotherapy in vivo with help of this vibrational spectroscopic sensor and find changes of water content in skin. We studied diffusion of dermatology drugs in vivo by FTIR ATR fiber sensor by tape removing of layers of stratum corneum cells. This ATR fiber sensor may have applications for detection of cancer tissue by vibrational spectroscopy.

8939-37, Session PSun

Quantitative analysis of melamine by multi-way partial least squares model with two-dimensional near-infrared correlation spectroscopy

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As extension of partial least square (PLS) to high order, multi-way partial least squares (N-PLS) incorporate the dependent variables in the decomposition of the independent variables. Therefore, N-PLS models have good ability of prediction. In this paper, a new approach for quantitative analysis of melamine in milk was proposed based on two-dimensional (2D) correlation near-infrared spectroscopy and N-PLS. The results demonstrated that the N-PLS model yielded relatively low the root mean square errors of prediction and the average relative prediction error as compared to PLS model. Therefore, N-PLS method was more robust for accurate quantification of the concentration of melamine in milk than PLS method. At the same time, this method can also be applied to other food safety detection areas.

8939-38, Session PSun

IR spectroscopic studies of intercellular liquid as tool for cancer detection

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Modern Fourier transform infrared (FTIR) spectroscopy is one of the candidate methods to improve efficiency of diagnosis of cancerous tissues. Biochemical changes that occur in the tissue cells usually are reflected in infrared spectral regions where spectral bands of proteins, lipids, nucleic acids and hydrocarbons are located. This technique is already successfully used for sectioned and dried tissue. Recent advances in FTIR spectroscopy, e.g., improvement of IR detectors as well as attenuated total reflection (ATR) accessories, allow obtaining infrared absorption spectra of intercellular liquid with high precision. The samples can be prepared by simple stamping of the tissue onto CaF2 substrate or an ATR prism.

By comparing infrared absorption spectra of normal and cancerous kidney intercellular liquid samples we found the spectral band at 1750 cm-1 (carbonyl stretch of lipids) to be the most suitable for cancer detection in the tissue, since it is weak in the spectrum of intercellular liquid of normal tissue while the corresponding band of intercullular liquid from cancerous tissue is much stronger. Such finding suggests that the liquid from cancerous tissue contains more lipids than from normal one. This observation could be explained from the biological point of view.

We found FTIR spectroscopy of the intercellular liquid to be sensitive, non-destructive and non-invasive diagnostic method, which can improve efficiency of diagnosis of cancerous tissues. The method has potential to be used (I) for chemical imaging of the tissue from which the intercellular liquid is taken and (II) in vivo for identification of the cancerous tissue areas during the surgery.

8939-39, Session PSun

Raman study of analysis for the states of maturation of neural cell

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Our purpose is to develop Raman spectroscopic technique as a new method for neuroscience. Micro-Raman spectroscopy was applied for analyzing molecular composition changes during maturation of neurons and monitoring drug treatment. Primary cultured neural cell was used for our studies as below.

(1) Analysis for neural maturation: The combination of confocal micro-Raman spectroscopy and partial least squares regression (PLSR) was carried out for prediction of the stages for maturation of neurons. Neural cell on culture dish constructs their networks and expresses new activities such as spontaneous activity. The construction of network system of the neural cells has been studied with an electrode array in the culture dish. Neurons reconstruct a network in the dish and generally have spontaneous activity after 10 days cultivation. The synchronization of the spontaneous activity takes place in the cultured neural cells after about 60 days. The matured neural cell network shows regulated pulsation with interval of several seconds without any stimulation. The spectra of live neural cells measured after 2, 8, 15, 30, 45, 60, 75 and 90 days of culturing are analyzed by PLSR.

(2) Drug response research: We investigated the possibility of Raman spectroscopy as a monitoring tool for drug response. Neurons were treated with cytosine arabinoside (Ara-C). This regent induces apoptosis for neurons by stimulation of p53 pathway. Ara-C treatment was performed to 3 days cultivated neurons for 24 hours. Raman measurement was carried out after 1, 7, 14 days from regent treatment.

8939-41, Session PSun

Raman spectroscopy study of thyroid tissues

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Raman spectroscopy has been successfully used for diagnose many different type of cancer reaching, in some cases, classification

efficiency up to 90% for ex vivo tumor and, for in vivo, online gastric cancer diagnosis, a sensitivity and specificity of 80.5% and 86.2%, respectively [1,2]. However, for thyroid disease, Raman spectroscopy has demonstrated a relatively poor discrimination. In our previous work classification between benign tissues (goitre and follicular adenoma) and malignant tissues (papillary and follicular carcinomas), the index was 72.5%, which we believe is mainly due to the tissue heterogeneity [3]. Therefore, the main goal of this work was to collect Raman data from a homogeneous thyroid tissue and compare the spectral characteristic with the histological image. For this end, several benign and malignant thyroid tissues were cut (Leica® Modelo CM 1100) into two subsequent slices and placed in the CaF2 windows. The first 5 µm slide were colored by HE to serve as a guide for Raman collection in the second 5 µm slide. A confocal Raman system (Rivers 3510) using a 785 nm laser was used. The discrimination index between benign and malignant tissues was 92%, which is much better than the previous results. In this way, we have shown that Raman spectroscopy can also be used to obtain valuable molecular information from different types of thyroid tissue, as well as, be able to reach a good classification index percentage for classification between benign and malignant tissues.

1. (Singh SP et al. J Cancer Res Ther. 2012 Jan;8 Suppl 1:S126-32. doi: 10.4103/0973-1482.92227)

2. (Shiyamala D et al. Journal of Biomedical Optics 17(8), 081418 (August 2012)

3. (Teixeira C. et al. Analyst 134 (2009) 2361-2370. doi:10.1039/b822578h)

8940-1, Session 1

Polarization-enhanced multispectral wide-field imaging for noninvasive in vivo assessment of collagen structures

Xin Feng, Rakesh Patel, Anna N. Yaroslavsky, Univ. of Massachusetts Lowell (United States)

In this study, we introduce a polarization enhanced multispectral wide-field reflectance imaging as a tool for noninvasive real-time monitoring collagen structure in vivo. Skin collagen is the major component of dermal structure and has been widely studied in the past. However, there are no technologies capable of quantitative noninvasive in vivo inspection of skin aging and structural changes over large fields and with sufficient resolution. Our in-house built imaging device is capable of rapid image acquisition in the spectral range between 390 nm and 750 nm with the field of view of ~ 3 cm ? 3 cm and lateral resolution of 13 μm. For quantitative assessment we evaluated collagen density and the size of individual collagen bundles. We have also determined and analyzed full width at half maximum (FWHM) of the intensity histogram and normalized average pixel values to further evaluate skin condition. Results obtained from 17 volunteers from 3 age groups showed decreased collagen density, decreased average pixel value and increased FWHM with age. The age-related collagen changes observed in our study are consistent with the results reported by other groups, obtained using immunohistochemistry and transmission electron microscopy. The results of our study indicate that polarization-enhanced multispectral imaging has potential for in vivo noninvasive real-time evaluation of human skin.

8940-2, Session 1

Monitoring hemoglobin concentration in normal and malignant oral tissues using diffuse reflectance spectroscopy (DRS): an in vitro study

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Oral cancer, a common dreaded disease which plays an important role in increasing mortality and morbidity rate higher in India and other countries. This may be due to the late stage diagnosis of the disease and also the sensitivity of the instrument is not up to the expectation. Over the few decades, Optical spectroscopy gains in more important in cancer diagnosis and aid the effective tool in early stage diagnosis. Among them, Diffuse Reflectance Spectroscopy (DRS) emerged as one of the diagnostic tools which can directly relates the absorption and scattering properties of tissues. As the Reflectance spectroscopy quantifies the changes in the tissues morphology, hemoglobin content, nucleus size and nucleus to cytoplasmic ratio directly, the biochemical and morphological changes in the malignant tissues can be accurately quantified using this technique. Many researchers applied this technique successfully in classification of normal to premalignant lesions.

In the present study, the Diffuse Reflectance Spectroscopy (DRS) of normal and malignant oral tissues were carried out using UV-VIS Spectrophotometer. The concentration of hemoglobin present in normal and malignant tissues were different when the normal tissues prognosis to malignant condition. In this context, it is aimed to quantify the hemoglobin content present in normal and malignant subjects and to study its statistical significancy to know whether there exists any diagnostic potential or not.

8940-3, Session 1

Multifocal non-contact setup for depth sensitive fluorescence imaging of early epithelial cancer

Caigang Zhu, Yi Hong Ong, Quan Liu, Nanyang Technological Univ. (Singapore)

Our previous Monte Carlo study has demonstrated that a lens based non-contact setup is able to achieve depth sensitive diffuse reflectance measurements on an epithelial tissue model in a point measurement setup. In this study, we expanded our point measurement system to an imaging setup to perform depth sensitive imaging on tissue phantoms in a large field of view. In the proposed setup, a micro-lens array was used to induce multi-focal illumination and a tunable lens was applied to image multiple foci into the tissue phantom at a range of depths. Another imaging lens was used to image diffusely reflected light into a 3-CCD camera. The tissue phantom represented a squamous cell carcinoma (SCC), which was made up of the top epithelial layer with a thickness of 680 μm and the bottom layer with a thickness of 1 cm. A cuboid phantom block representing an early SCC tumor with a thickness of 340 μm was buried inside the epithelial layer. Depth sensitive images acquired from the tissue phantoms demonstrated the change in the contrast around the boundary between the epithelial layer and the tumor regions as the focal depth of the tunable lens changed. This was further confirmed by the changes in the reconstructed diffuse reflectance spectra. Our study demonstrated that a non-contact setup could be used to perform depth sensitive imaging on epithelial tissues for the diagnosis of early epithelial cancer.

8940-4, Session 1

Experimental methods for recording stable NIRS measurements from upright alert infants

Ashley Cannaday, James Goodwin, Brooke D. Beier, Andrew Berger, Univ. of Rochester (United States)

Near-infrared spectroscopy (NIRS) is a widespread technique for the non-invasive study of cerebral hemodynamics in alert humans. Many groups, including our own, have successfully detected stimulation-related responses from alert adults and children, clearly indicating regions of activation in the brain. The NIRS modality is particularly attractive for studying cognitive development in infants, with potential clinical applications. Months-old infants present singular problems as subjects, however, because they cannot be instructed to hold still or to pay attention. Most NIRS studies on three- to nine-month-old infants report having to reject approximately 50% of measurement trials due to motion artifacts and subject non-compliance. We describe several modifications that address motion/compliance issues and thereby improve NIRS measurements from upright and alert infants. First, the NIRS headpiece is reconfigured with fewer optical fibers to reduce inertia, increase comfort, and improve conformity to the head; at the same time, however, fiber density over the sampled region remains dense to avoid missing the activation. Second, the visual-stimulation protocol is altered

significantly to keep the attention of the infants focused on the screen throughout the measurement sequence. Lastly, optical signal strength is screened at the outset of each trial, enabling a quick determination of whether the probe position needs adjustment. With these revisions to the experimental process, we have significantly reduced the typical motion artifacts seen during trials of alert, upright infants, and we obtain visual activation signals from most infants.

8940-5, Session 1

Propagation and scattering of complex structured light in turbid scattering medium

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Polarization is one of light's most salient features, even more so than its spectral or coherence properties. When light interacts with the matter its state of polarization is changed. The state of polarization of "simple" linearly, elliptically or circularly polarized light has long (since 1800s) been used to characterize material surfaces, thin films and transparent media. The structure of light can be more "complex" in addition to the conventional state of polarizations, the light beams can be radially or azimuthally polarized and carry orbital angular momentum. When complex structured light propagates in a homogeneous transparent medium, both spin and orbital angular momentum are conserved. In the medium with anisotropic scattering the spin or angular momentum are changed that leads to spin-orbit interaction. Such a spin-orbit interaction leads to the mutual influence of the polarization and the trajectory of the light propagation. A Monte Carlo based model is presented and the results of simulation of complex structured light propagation in turbid tissue-like media with a primary goal to proof the concept of using structured light for tissue diagnosis. The propagation of various beams are considered and compared, including linear, elliptically and circularly polarized, as well as radial and azimuthally polarized cylindrical vector beams on the higher order Ponicare sphere(HOPS).

8940-6, Session 1

Diffusing-wave polarimetry for tissue diagnostics

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Bearing in mind the basic polarization formalism and coherent properties of light, we discuss the peculiarities of circularly polarized light in tissue-like turbid media. We exploit the directional awareness of circularly polarized light propagating in media that exhibit strong multiple scattering, and investigate its use for characterizing the anisotropy of scattering particles. Considering the propagation of light through a medium where multiple scattering events occur, light backscattered an odd number of times will correspond to a reversal in helicity relative to the incident source, and thus, contribute the cross-polarized portion of the detected signal. Whereas, light experiencing an even number of backscattering events contributes to the co-polarized signal. The developed phenomenological model is shown to have excellent agreement with the experimental data, and with the results obtained by the polarization tracking Monte Carlo modeling. By analogy to diffusing-wave spectroscopy we call this approach diffusing-wave polarimetry, and illustrate its utility in probing cancerous and non-cancerous tissue samples.

8940-7, Session 2

Nonlinear microspectroscopy: a tool to discriminate between healthy skin and nonmelanoma skin cancer

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In this contribution the utility of non-linear optical microscopy to investigate alterations of the human skin and in particular non-melanoma skin cancer (NMSC) – the most frequently occurring malignant neoplasm – is outlined.

Ex vivo sections of (NMSC) are investigated using a combination of coherent anti-Stokes Raman scattering (CARS), second harmonic generation (SHG) and two photon excited fluorescence (TPEF) - referred to as multimodal imaging. By tuning the CARS process to be sensitive to the C H symmetrical stretching vibration, CARS yields the spatial distribution of lipids. TPEF – on the other hand – visualizes the spatial distribution of the skin's endogenous fluorophores. Finally, SHG delineates structures lacking inversion symmetry, i.e. in particular collagen fibers. Combined, multimodal imaging provides in-depth information on different levels of tissue organization, i.e., molecular groups, fluorophores and supramolecular structures.

To evaluate NMSC first the appearance of healthy skin in multimodal imaging is characterized. On these grounds NMSC was analyzed. As a result NMSC tumor islands show a relatively increased TPEF intensity and lack SHG signal. Moreover, the most frequently occurring two subtypes of NMSC, i.e., basal cell carcinoma (BCC) and the more aggressive but less abundant squamous cell carcinoma (SCC) can be distinguished by evaluation of CARS images. The later shows increased CARS signal compared to the surrounding collagen containing tissue, while the tissue of the BCC displays a relatively decreased CARS signal intensity. Besides the discrimination of NMSC, multimodal imaging provides prognostic relevant information. Hallmarks of cancer like the bioenergetics, the tumor-stroma interaction as well as the fat metabolism are readily evaluated. In summary, non-linear microscopy provides means for the localization, classification and prognosis of NMSC.

This work was financially supported by the 'Europäischer Fonds für Regionale Entwicklung (EFRE)', the 'Thüringer Ministerium für Bildung, Wissenschaft und Kultur' (TMBWK, projects: B578-06001, 14.90 HWP and B714-07037), the European network of excellence p4l (photonics4life), the Federal Ministry of Education and Research, Germany, (FKZ: 13N10508).

8940-8, Session 2

Detecting breast cancer in single cells

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Great advances have been made in the early detection and treatment of breast cancer, but it is still difficult for pathologists to identify single cancer cells. We have established the feasibility of using fluorescence and fluorescence polarization of intravital dye, methylene blue (MB), for detecting breast cancer in excisions. The goal of this work was to investigate if by monitoring fluorescence and fluorescence polarization of MB we may able to distinguish cancer cells from normal. Fluorescence

and fluorescence polarization is sensitive to the changes in biochemical and biophysical properties of the environment of the fluorophore. Cancer cells have higher cell division rate than normal cells, which may leads to differences in cell membrane fluidity, intracellular viscosity and dye localization within the cells. In this study, we used multimodal confocal microscopy to investigate methylene blue stained mouse embryonic fibroblast cells (3T3) and metastatic breast cancer cells (4T1). Confocal reflectance, fluorescence and fluorescence polarization images were acquired and fluorescence polarization values were calculated for both cancer and normal cells. Our results showed that fluorescence polarization of breast cancer cells is 30% higher than that of normal cells. Our preliminary results suggest that detection of changes in fluorescence polarization may allow for discrimination of cancer cells reliably and rapidly. Multimodal confocal imaging shows promise for monitoring cancer induced changes on the single cell level.

8940-9, Session 2

Automated cellular pathology in noninvasive confocal microscopy

Monica Ting, James Krueger, Daniel S. Gareau, The Rockefeller Univ. (United States)

This project developed a computer algorithm to automatically identify and count melanocytes and keratinocytes in 3D reflectance confocal microscopy (RCM) images of the skin. Currently there is lack of an automated pathological system on the confocal microscope to locate and count cells. If successful, such a system would increase understanding of and enable prevention of superficial spreading melanoma (SSM). The process was divided into two main parts: machine learning and implementation. Machine learning involved looking at the images to measure the size of cells through a 2-D Fourier transform and developing an appropriate mask with the erf() function to model the cells. Implementation involved processing the images to identify cells whose image segments provided the least difference when subtracted from the mask. With further simplification of the algorithm, the program may be directly implemented on the RCM images to indicate the presence of keratinocytes in seconds and to quantify the keratinocytes size in the en face plane as a function of depth. Using this system, the algorithm can identify any irregularities in maturation and differentiation of keratinocytes, thereby signaling the possible presence of cancer.

8940-10, Session 2

Classifying collagen remodeling and pathological lipid deposition by multimodal label-free microscopy and image statistics

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In this study we present a novel image analysis methodology to quantify and to classify morphological details in tissue collagen fibril organization and lipid deposition. Co-localized collagen (second harmonic, SHG) and lipid (coherent Raman, CARS) images of atherosclerotic artery walls were acquired by a supercontinuum-powered multi-modal nonlinear microscope. Textural features based on the first-order statistics (FOS) and gray level co-occurrence matrix (GLCM) parameters were extracted from the SHG and CARS images. Multi-group classifications based on support vector machine of SHG and CARS images were subsequently performed to investigate the potential of texture analysis in providing quantitative descriptors of structural and compositional changes during disease progression. Using a rabbit model, different collagen remodelling and lipid accumulation patterns in disease tissues can be successfully tracked using these image statistics, thus providing a robust foundation for classification. When the variation of the CARS image features were tracked against the age of the rabbit, it was noticed that older animals (advanced plaques) present a more complex necrotic core containing high-lipid extracellular structures with various shapes and distribution. With combined FOS and GLCM texture statistics, we achieved reliable classification of SHG and CARS images acquired from atherosclerotic arteries with >90% accuracy, sensitivity and specificity. The proposed image analysis methodology can also be applied in a wide range of applications to evaluate conditions involving collagen remodelling and prominent lipid accumulation.

8940-11, Session 3

Brain Metastasis Detection by Resonant Raman Optical Biopsy Method

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Resonant Raman (RR) spectroscopy can cause the enhancement of Raman signal from particular bonds associated with key molecules due to changes at molecular level. In this study, RR is used for detection of human brain metastasis in ex-vivo from five kinds of primary organs (lung, breast, kidney, rectal and orbital adenocarcinoma). Totally fourteen specimens were investigated using a micro-confocal Raman system with excitation wavelength of 532 nm.

The RR enhanced peaks and the peak changes in intensities were found from all the brain metastases tissues. The RR spectra demonstrated the enhancement for amide II band, , but decreasing DNA band, lipids and proteins at 2885 cm-1 and 2934 cm-1 in all the brain carcinoma tissues. The results of RR spectra from carcinoma tissues were compared with those of normal brain tissue and primary cancerous tissues. Analysis of RR spectra combined with the results of histopathology and immunohistochemistry (IHC) reveal the differences between cancerous and normal brain tissues. The theory of Naive Bayes classifier was used to classify a set of RR spectra of brain metastasis of primary lung cancer tissue and normal brain tissue, yielding diagnostic sensitivity and specificity at 99.9% and 100%, respectively. This indicates that the RR spectroscopic technique demonstrate high accuracy and reliability; therefore, it may provide new molecular-based optical tools for diagnosis and classification of brain metastatic cancer.

8940-12, Session 3

Raman microspectroscopic study of oral buccal mucosa

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Oral cancer is the most common cancer among Indian males, with 5-year- survival-rates of less than 50%. Efficacy of Raman spectroscopic methods in non-invasive and objective diagnosis of oral cancers and

confounding factors has already been demonstrated. The present Raman microspectroscopic study was undertaken for in-depth and site-specific analysis of normal and tumor tissues. 10 normal and 10 tumors unstained sections from 20 tissues were accrued. Raman data of 160 x 60 ?m and 140 x 140 ?m in normal and tumor sections, respectively, were acquired using WITec alpha 300R equipped with 532 nm laser, 50X objective and 600 gr/mm grating. Spectral data were corrected for CCD-response, background. First-derivatized and vector-normalized data were then subjected to K-mean cluster analysis to generate Raman maps and correlated with their respective histopathology. In normal sections, stratification among epithelial layers i.e. basal, intermediate, superficial and stroma was observed. Tumor, stromal and inflammatory regions were identified in case of tumor section. Extracted spectra of the pathologically annoted regions were subjected to Principal component analysis. Findings suggest that all three layers of normal epithelium can be differentiated against stroma. And epithelium, basal and superficial layers can be separated while intermediate layer show misclassifications. In tumors, discrimination of inflammatory regions from tumor cells and tumor-stroma regions were observed. Spectra of tumor regions can be classified from normal spectra of different regions – basal, superficial, stroma. Finding of the study indicate Raman mapping can lead to molecular level insights of normal and pathological states.

8940-13, Session 3

In vivo Raman spectroscopy of cervix cancers

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Cervix-cancer is the third most common female cancer world-wide. It is the leading cancer among Indian females with more than million new diagnosed cases and 50% mortality, annually. The high mortality rates can be attributed to late diagnosis. Efficacy of Raman spectroscopy in classification of normal and pathological conditions in cervix cancers on diverse populations has already been demonstrated. Our earlier ex vivo studies have shown the feasibility of classifying normal and cancer cervix tissues as well as responders/non-responders to Concurrent chemoradiotherapy (CCRT). The present study was carried out to explore feasibility of in vivo Raman spectroscopic methods in classifying normal and cancerous conditions in Indian population. A total of 100 normal and 79 tumor in vivo Raman spectra, from 63 subjects, were recorded using a fiberoptic probe coupled HE-785 spectrometer, under clinical supervision. Spectra were acquired for 5 s and averaged over 3 times at 80 mW laser power. Spectra of normal conditions suggest strong collagenous features and abundance of non-collagenous proteins and DNA in case of tumors. Preprocessed spectra were subjected to Principal Component-Linear Discrimination Analysis (PC-LDA) followed by leave-one-out-cross-validation. Classification efficiency of ~99% and 91% for normal and cancerous conditions respectively, were observed. Findings of the study corroborates earlier studies and suggest applicability of Raman spectroscopic methods in combination with appropriate multivariate tool for objective, noninvasive and rapid diagnosis of cervical cancers in Indian population. In view of encouraging results, extensive validation studies will be undertaken.

8940-14, Session 3

Fast reconstruction of Raman spectra from wide-band measurements of Raman signals with fluorescence background

Shuo Chen, Xiaoqian Lin, Quan Liu, Nanyang Technological Univ. (Singapore)

Raman spectroscopy has demonstrated great potential in clinical and biomedical applications. But slow data acquisition due to weak Raman signals has prevented its use in measuring events varying with time, especially in an imaging setup. Our previous study has shown that the Raman imaging can be realized by the reconstruction of Raman spectra from wide-band measurements of Raman signal without fluorescence background. Although there are several techniques that can minimize fluorescence background in Raman measurements, additional and complex equipment is always needed. In this study, we investigate the fast reconstruction of Raman spectra from the wide-band measurements of Raman signals with fluorescence background. The reconstruction method is evaluated on both spontaneous Raman data and surface enhanced Raman spectroscopy (SERS) data. The results show high accuracy in reconstructed Raman spectra, especially for SERS, compared to measured Raman spectra. This method opens a new revenue for using Raman imaging to investigate fast changing phenomena in biological samples.

8940-15, Session 3

Characterization of urine of normal subjects and oral cancer patients by Raman spectroscopy

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Urine is considered as one of the diagnostically important bio fluids, as it has many metabolites. The distribution and the physiochemical properties of the metabolites may vary during any metabolic change and different pathologic conditions. Among various optical spectroscopic techniques raman spectroscopy has been emerged as a tool in identifying several diseased conditions, including oral cancers .This is because, it can measure the spectra for each biological molecule and this can facilitate to find the exact molecular composition of the urine. In this study, we aimed at characterising the urine of both normal and patients with oral cancer using Raman spectroscopy to identify the differences in the chemical composition of urine between them. Principal component analysis based Linear discriminant analysis were also made to discriminate the cancer patients from normal subjects. The details of the Raman spectral signatures of urine samples between normal and cancer patients and its statistical significance will be discussed.

8940-16, Session 4

Autofluorescence lifetime metrology for label-free detection of cartilage matrix degradation

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Proteolytic degradation of cartilage extracellular matrix (ECM) is the hallmark of arthritis that leads to joint destruction. Detection of early biochemical changes in cartilage before irreversible structural damages become apparent is highly desirable. To this end we are investigating the potential of autofluorescence lifetime (AFL) to provide a label-free read

out of cartilage ECM degeneration in arthritis. The fluorescence lifetime of cartilage ECM is an intrinsic characteristic, which can be modified by changes in the local chemical or physical environment of the fluorophore. Here we present ex vivo experiments demonstrating that degradation of cartilage ECM does affect AFL of cartilage tissue but not its emission spectrum.

A compact fibre-optic probe-based multidimensional fluorometer utilising ultraviolet excitation at 355 nm or 375 nm was used to make single point spectrally-resolved lifetime measurements of porcine articular cartilage explants treated with different proteinases. Differences in cartilage AFL were also visualized by fluorescence lifetime imaging microscopy (FLIM) using a wide-field fluorescence microscope employing excitation at 365 nm provided by a frequency-doubled Ti:Sapphire laser. Degradation of cartilage matrix components by treating with retinoic acid or digesting with trypsin, bacterial collagenase, or matrix metalloproteinase 1 resulted in significant reduction of AFL of the cartilage in both a dose and time dependent manner. Differences in cartilage AFL were confirmed by FLIM microscopy. Thus our data suggests that AFL of cartilage tissue is a potential non-invasive readout to monitor cartilage matrix integrity for diagnosis of arthritis as well as monitoring the efficacy of anti-arthritic therapeutic agents.

8940-17, Session 4

Experimental validation of Monte Carlo modeling of depth sensitive fluorescence illumination and detection configurations in skin tissues

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Conventional fluorescence spectroscopy of subsurface tissue implemented by microscope objective lens suffers from limited depth sensitivity due to the dominance of undesired fluorescence signals from the overlaying tissues. Furthermore, the need of altering the sample-probe distance to achieve different focal depths renders this technique inconvenient for clinical applications involving in vivo measurements. Fiber based contact setup can suppress near surface signals by changing the distance between the excitation and collection fibers but the contact with the sample introduces uncertainty due to inconsistent probe-sample pressure. Recently, a novel non-contact optical configuration has been proposed by our group that demonstrated improved depth sensitivity to subsurface layer in a turbid medium. This setup employed a cone shell illumination and collection configuration implemented by combination of axicon lenses. In this study, we present the first flexible Monte Carlo method to model non-contact fluorescence spectroscopy in a lens based setup. The model was able to create the cone and cone shell configuration in both the illumination and collection geometry using a conventional microscope objective lens or an axicon lens. The depth sensitivity of this method to the epidermis of different thicknesses in a skin model was studied by A) using either the cone or cone shell setup in both the illumination and collection geometry B) using the cone setup in illumination geometry and the cone shell setup in the collection geometry or the opposite way. These simulated results were validated with the corresponding experimental results. The model clearly indicates that a cone shell-cone shell geometry, implemented with axicon lens, which was implemented in the earlier experiments, would yield the largest range of depth sensitivity among all four illumination and collection geometries.

8940-18, Session 4

Cervical precancer detection with polarized light based hand held device

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A prototype device (hand held probe) has been designed, fabricated and automated to obtain the intrinsic fluorescence from human cervical tissue samples by measuring the polarized fluorescence and polarized elastic scattering. This is based on the fact that in a turbid medium such as the biological tissue, the intrinsic fluorescence gets strongly modulated by the interplay of scattering and absorption. This masks valuable biochemical information which is present in the intrinsic fluorescence. These distortion effects can be reduced by use of a method which is based on simultaneously acquired polarized fluorescence and polarized elastic scattering spectra from a turbid medium. The polarized fluorescence spectra are normalized by the polarized elastic scattering spectra. Intrinsic fluorescence of NADH and collagen, which are the dominant fluorophores of the epithelium and stroma, respectively, of cervical tissue has earlier been extracted using 325nm and 370nm wavelength. However the resultant spectra exhibit contribution from both fluorophores and this has affected the discrimination sensitivity and specificity of normal and precancerous tissues. With 405nm excitation wavelength, however, FAD contribution is most dominant in the fluorescence spectra and improves discrimination efficiency significantly. The sensitivity and specificity for discrimination are found to be 100% and 90 % respectively. The hand held device built in-house has been used in these measurements and shows promise as a useful tool for in vivo cervical precancer detection by polarized fluorescence.

8940-19, Session 4

Stoke's shift spectral features of blood plasma and urine of bladder cancer patients

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The gold standard for diagnosing bladder cancer is biopsy obtained during cystoscopy which is painful and expensive. In the current study, we had explored the potential of stokes shift spectra (SSS) of blood plasma, and urine samples for diagnosis of bladder cancer. For this purpose, blood and urine samples from healthy and bladder cancer individuals (N=30) were collected . The blood samples, collected in EDTA vials, were centrifuged to get supernatant plasma; the first voided urine samples of 20 ml were collected in sterile plastic container and both types of samples were kept in refrigerator and analyzed within four hours by SSS.

The spectral features are interpreted on the basis of the relative intensity of fluorescence bio markers like tryptophan, tyrosine, collagen, elastin, and flavin found in their blood plasma, and urine samples. The characteristic spectral feature of bladder cancer plasma and urine is the appearance of a new band at 315 nm (most likely due to structural protein collagen). It is not manifested in any other cancer, and never in normal controls .So , this technique could be of significant clinical value for repeated monitoring of progression or regression of disease .

8940-20, Session 4

Noninvasive diagnosis of oral cancer by Stokes shift spectroscopy

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The objective of this study is to evaluate the diagnostic potential of stokes shift (SS) spectroscopy (SSS) for detection and characterization

of normal, precancer and cancerous oral lesions in vivo. The SS spectra were measured by simultaneously scanning both the excitation and emission wavelengths while keeping a fixed wavelength interval delta lambda = 20 nm between them. The SSS technique simplifies emission spectrum and provides more sharp spectral signatures of the endogenous fluorophores in the tissues. The SS spectra from oral buccal mucosa were recorded in the 250 – 600 nm spectral range from 20 sites of 10 healthy volunteers and 20 sites of 20 patients with different pathological oral abnormalities. The SS spectral data were categorized based on pathological reports as normal (n=20), precancer (n=10), and cancer (n=10). Characteristic, highly resolved peaks and significant spectral differences between normal and different pathological oral tissues were observed. The observed spectral peaks were tentatively identified from measured SS spectra of standard fluorophores. The SS spectra of normal, precancer and cancerous oral tissues revealed distinct peaks around 300, 355, 395, and 420 nm which are attributed to tryptophan, collagen, and NADH respectively. Using SSS technique one can obtain the key fluorophores in a single scan and hence they can be targeted as a tumor markers in this study. To quantify the observed spectral differences between normal and different pathological oral tissues were verified by statistical and ratio parameter analysis. The result of the current study has the potential for diagnosis and classification of oral lesions noninvasively.

8940-21, Session 4

Fluorescence spectroscopy of tongue malignancy

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Although considerable progress has been made in understanding the cellular and molecular mechanisms of tumorigenesis, the lack of appropriate diagnostic methods for early diagnosis leading to delay in therapy has lead to increase in the mortality rate in various types of cancers. These require experienced pathologists, and usually have large false negative results because of sampling errors, fatigue factor in examination of large number of slides, inexperience of Pathologist etc. One of the important factors for successful therapy of any malignancy is early diagnosis. Spectroscopy techniques are extremely sensitive for the analysis of biochemical changes in the cellular systems. Laser spectroscopy techniques have high specificity and sensitivity compared to conventional methods. The methods are particularly suitable for early detection because biomolecular changes precede any disease, whereas histo-morphological changes used in pathology take place towards later stages.

This work deal with in vivo fluorescence measurement of tongue malignancy using our home assembled LIF system. About 892 fluorescence spectra from tongue (top, tip, bottom and lateral) have been recorded from 330 subjects under normal (133), premalignant (154) and malignant (63) condition, by excitation with 325 nm CW He-Cd laser. Tongue top, tongue tip, tongue lateral, and bottom give spectra differing from each other. Under clinically normal, pre-malignant and malignant conditions the fluorescence spectra are found to be noticeably different. The analyses results (Principal Component Analysis) of the data have shown very good sensitivity and specificity to diagnose tongue malignancy.

8940-39, Session PTue

Enhancing the depth of tissue microscope imaging using two-photon excitation of the second singlet state of fluorescent agents

Yang Pu, Lingyan Shi, The City College of New York (United States); Sebastião Pratavieira, Univ. de São Paulo (Brazil); Robert R. Alfano, The City College of New York (United States)

One of the goals of biomedical optical community is to increase the imaging depth for optical tissue microscopy. Choosing the appropriate imaging wavelength according to light attenuation caused by tissue provides a means of increasing the imaging depth. There is an “optical window” for biological tissues in the far-red to near infrared (NIR) range (650 nm - 1,100 nm), which allows light to penetrate deep into tissue.

By exciting the first singlet (S1) state of intrinsic or extrinsic fluorophores in the visible range, two-photon microscope (2PM) techniques have made a significant contribution to enhance optical imaging depth over conventional fluorescence microscope. The penetration depth of current 2P imaging techniques are still limited by the scattering of the operating light in the visible range and re-absorption of the emitted light. To achieve deeper 2P imaging, the 2P excitation of the second singlet (S2) state of a group of fluorescent agents with near infrared emission, e.g. Chlorophyll a (Chl a) and Indocyanine green (ICG), is used to make both 2P excitation and emission falling in near infrared (NIR) “tissue optical window”. The key underlying mechanism behind the success of using S2 as the pumping level is the rapid nonradiative relaxation from S2 to S1. The 2P pumping the S2 state of Chl a and ICG was investigated to demonstrate for the first time that this simple but innovated and efficient approach can be used to enhance imaging depth.

8940-40, Session PTue

Spatial Fourier frequency statistics analysis of human cervix precancer tissues

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It is important to detect cervical dysplasia, Cervical Intraepithelial Neoplasia (CIN). CIN is the potentially premalignant and abnormal squamous cells on surface of cervix. In this study, the spatial frequency spectra of pre-cancer cervical tissues is used to detect differences among different grades of human cervical tissues. Seven sets of thick tissue sections of human cervix of normal, CIN 1, CIN 2, and CIN 3 tissues are studied. The confocal microscope images of the stromal region of normal and CIN human tissues were analyzed using Fast Fourier Transform (FFT) to generate the spatial spectra. It is observed that higher frequency components exist in CIN tissues than those in normal tissue, as well as those in higher grade CIN tissue than those in lower grade CIN tissue. The width of the spatial frequency of different types of tissues is used to create a criterion for CIN grading by training a support vector machine classifier (SVM). The results show that the randomness of tissue structures from normal to different stages of precancer in cervical tissue can be recognized by fingerprints of the spatial frequency. The efficacy of spatial frequency analysis for CIN grading is evaluated as excellent since high AUC (area under the ROC curve), sensitivity and specificity are obtained by the statistics study. This works lays the foundation of using FFT analysis of spatial frequency spectra for a histology evaluation.

8940-41, Session PTue

Investigation of relative content of tryptophan for monitoring breast cancer aggressiveness by native fluorescence spectroscopy

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Previous studies of optical sensing cancer have shown that native fluorescence spectra can be used to distinguish cancer from normal tissues and cells. The native fluorophores, such as NADH and FAD, are involved in the oxidation of fuel molecules. 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 amino acid tryptophan is needed for cancers to progress. The elevated levels of tryptophan are expected to help tumors evolution.

The aim of the present research is to determine if the native fluorescence spectroscopy can be utilized to detect changes of the key fluorophore compositions related to different types of breast cancer cell lines with different risk levels. Different types of cancer cell lines with different risk levels, such as primary tumor carcinoma (MCF-7), and advanced metastatic aggressive (MDA-MB-231) cell line, as well as normal cell lines (fibroblast), were excited by the selective wavelength of 300 nm. The contributions of principal biochemical components to fluorescence spectra from the cell samples were investigated using the different non-negative constraint blind source separation methods. The higher relative content of tryptophan is observed in the aggressive cancer cell lines in comparison with the non aggressive cell lines. This work shows that the changes of relative contents of tryptophan over NADH obtained by native fluorescence spectroscopy offer a potential criterion - for detecting breast cancer cell lines with different risk levels.

8940-42, Session PTue

Tumor margin detection using optical biopsy techniques

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A method is sorted to accurately determinate the margin assessment. The location of the tumor margin is an important factor to predict the outcomes of surgery and therapy. Currently, there is a lack of technique to accurately assess margins for breast and gastrointestinal carcinoma in vivo. In this report, the cancerous margin was detected using optical biopsy techniques of resonance Raman (RR) spectroscopy techniques, as well as using the results obtained from fluorescence spectroscopy techniques, which has potential for in vivo study.

This study is focused on human breast and gastrointestinal (GI) carcinoma including gastric, rectal, and colonic organs. The pairs of cancerous and normal tissue samples were taken from a same patient along with the medical reports. The measurements were taken by scanning lesion from center to around 2 to 10.7 mm region to find the changes of RR spectra between cancerous and normal tissues in the molecular level. The sharp margin of the tumors was found by the changes of RR spectral peaks within 2 mm distance. This result was verified using fluorescence spectra with 300 nm, 320 nm and 340 nm excitation, in a typical specimen of gastric cancer tissue within a positive margin in comparison with normal gastric tissues. Bayesian statistical theory was performed on the RR spectral data yielding a predictions accuracy rate of 0.93 verified by clinical histopathology and immunohistochemistry reports (gold standard). This study demonstrates the potential use of RR and fluorescence spectroscopy techniques as new approaches to determine the margin assessment.

8940-22, Session 5

Quantitative photonic pathology for cancer diagnosis and prognosis

Michael Reilly, Kyle Scherer, Fairfield Univ. (United States); Yongchao Ge, Mount Sinai School of Medicine (United States); Jonathan Melamed, New York Univ. Langone Medical Ctr. (United States); Min Xu, Fairfield Univ. (United States)

A recent trend in optical biopsy is to realize label-free quantitative pathology for cancer diagnosis and prognosis based on light-tissue interaction. Such an approach may avoid time consuming tissue processing, achieve real time diagnosis, and overcome the subjective nature of the conventional histopathological analysis.

We present here our effort in developing label-free quantitative photonic pathology combining quantitative phase imaging (QPI) and tissue native fluorescence spectroscopy for cancer diagnosis and prognosis. This quantitative photonic pathology is implemented on an epi-fluorescence and differential phase contrast (DIC) microscope and can be used to image intact fresh tissue specimens or unstained pathology slides. The absolute concentration and 2D distribution of major native fluorescent molecules, including tryptophan, NADH, FAD and porphyrin, the optical phase delay map, and the light scattering characteristics maps are extracted from tissue native fluorescence and DIC images. A risk score is then computed from these co-registered maps reflecting tissue morphology and metabolomics to predict cancer grade and prognosis. This quantitative photonic pathology is used to examine over 150 cases of prostate tissue specimens of varying cancer grades and prognostic outcomes. Our preliminary results suggest that a high accuracy (>80% with ~80% sensitivity and ~80% specificity) for both diagnostic and prognostic purposes can be achieved. Work is in progress to improve its performance further.

8940-23, Session 5

Quantitative wavelength-dependent measurement of contrast in NIR and extended NIR spectral range (650-1500 nm) in biological phantoms

Daniel Salo, David M. Kim, Mikhail Y. Berezin, Washington Univ. School of Medicine in St Louis (United States)

The penetration depth of light in biological tissue circumscribes the progression of optical imaging. In order to identify the optimal wavelength for the highest contrast, we explored the optical contrast as a function of depth and wavelengths of excitation in a broad spectral range. Our customized optical hardware featuring a scanning microscope fiberoptically connected to an imaging spectrograph equipped with silicon and InGaAs CCD diode array detectors allowed directed measurement the intensity of NIR (650-950 nm) and exNIR (950-1500) light transmitted through emulsified colloids and tissue phantoms. We demonstrated that the contrast depends on the phantom type, its thickness and the wavelength. By comparing the corresponding contrast values exNIR light at certain wavelengths provides better contrast than NIR light in intralipid phantoms thicker than 4.5 mm and in tissue phantoms (chicken breast) thicker than 1.5 mm. Our results suggest that distinguishing biological features below a certain depth may benefit from the application of the exNIR for in vivo. The potential applications of exNIR in tissue biopsy are discussed.

8940-24, Session 5

Parametric study of different contributors to tumor thermal profile

Michal Tepper, Israel Gannot, Tel Aviv Univ. (Israel)

Treating cancer is one of the major challenges of modern medicine. There is great interest in assessing tumor development in in vivo animal and human models, as well as in in vitro experiments. Existing methods are either limited by cost and availability or by their low accuracy and reproducibility. Thermography has the potential of being a non-invasive, low-cost, non-radiative and easy-to-use tumor monitoring method. Tumors can be detected by thermal images due to their relatively higher or lower temperature compared to the temperature of the surrounding healthy tissue. Extensive research was performed to show the validity of thermography as an efficient method for tumor detection and the possibility of extracting tumor properties from thermal images, showing promising results. However, deducing from one type of experiment to another is difficult due to differences in tumor properties, especially between different types of tumors. There is a need for a research linking different types of tumor experiments.

In this research, parametric analysis of possible contributors to tumor thermal profiles was performed. The effect of tumor geometrical, physical and thermal properties was studied, both independently and together, in phantom model experiments and in computer simulations. Theoretical and experimental results were cross-correlated to validate the models that were used and to increase the accuracy of the simulated complex tumor models. The contribution of different parameters in various tumor scenarios was estimated and the implication of these differences on the observed thermal profiles was studied. The correlation between animal and human models will be extensively discussed.

8940-25, Session 5

Optical characterization of ex-vivo axillary lymph nodes of breast-cancer patients using a custom-built spectrophotometer

Ashwin Sampathkumar, Riverside Research Institute (United States); Emi Saegusa-Beercroft, Univ. of Hawai'i Kuakani Medical Ctr. (United States); Jonathan Mamou, Parag V. Chitnis, Riverside Research Institute (United States); Junji Machi, Department of General Surgery, University of Hawai'i and Kuakani Medical Center (United States); Ernest J. Feleppa, Riverside Research Institute (United States)

Quantitative Photoacoustics is emerging as a new hybrid modality to investigate diseases and cells in human pathology and cytology studies. Optical absorption of light is the predominant mechanism behind the photoacoustic effect. Therefore, a need exists to characterize the optical properties of specimens and to identify the relevant operating wavelengths for photoacoustic methods. We have developed a custom low-cost spectrophotometer to measure the optical properties of human axillary lymph nodes dissected for breast-cancer staging. 34 lymph nodes obtained from 13 breast-cancer patients were optically measured using our system. 7 nodes were positive for metastatic breast cancer and 27 were cancer free. Optical extinction curves of positive and negative nodes were determined in the spectral range of 400 to 1000 nm. Clinical protocol dictates that the lymph node specimens be preserved in saline bath for pathological studies. This translates to a scenario where the specimens are saturated with saline and osmotic perfusion of saline into the specimen takes place. We have developed a model to estimate the tissue optical properties taking into account the role of fat in the osmotic intake of saline into the dissected nodes. Our results enabled us to select the optimal optical wavelengths for maximizing the contrast between metastatic and noncancerous nodes in planned photoacoustic experiments. In the long-term, understanding optical properties of lymph nodes will help us to provide a personalized light-based diagnostic and therapy procedure for each patient, based on their medical history.

8940-26, Session 5

Enhanced visualization of the bile duct via parallel white light and ICG fluorescence laparoscopic imaging

Stavros G. Demos, Lawrence Livermore National Lab. (United States); Shiro Urayama, UC Davis Health System (United States)

Despite best efforts, bile duct injury during laparoscopic cholecystectomy is a major potential complication. Precise detection method of common bile duct during laparoscopic procedures would minimize the risk of injury. Towards this goal, we have developed a compact imaging instrumentation designed to enable simultaneous acquisition of conventional white color and NIR fluorescence endoscopic/laparoscopic imaging using ICG as contrast agent. The capabilities of this system, which offers optimized sensitivity and functionality, are demonstrated for the detection of the bile duct in an animal model. This design could also provide a low-cost real-time surgical navigation capability to enhance the efficacy of a variety of other image-guided minimally invasive procedures.

8940-27, Session 5

Time-resolved fluorescence imaging to characterize the cancer specific biomarkers

Yasaman Ardeshirpour, Victor Chernomordik, National Institutes of Health (United States); Moinuddin Hassan, National Institute of Child Health & Human Dev. (United States); Rafal Zielinski, Jacek Capala, National Cancer Institutes (United States); Amir Gandjbakhche, National Institute of Child Health & Human Dev (United States)

Current goal in cancer treatment is to find the cancer causing biomarkers and deactivate or downregulate those biomarkers by targeted therapeutic agents (e.g. monoclonal antibodies) to suppress the tumor growth and its metastasis.

Detection of those biomarkers is mainly semi-quantitative and based on ex-vivo methods such as as immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH). These methods are all invasive and require biopsies from tissue specimens. However, the number of times that biopsies can be taken is limited and due to heterogeneity of the tumor, it is hard to characterize the tumor by small number of biopsy samples. Therefore, there is a high demand for real-time in-vivo imaging techniques to detect and monitor the expression of cancer biomarkers before and during the course of therapy, especially at the early stages of the treatment.

Time resolve fluorescence imaging has the information of fluorescence lifetime in addition to fluorescence intensity. We will discuss the information that can be obtained by time resolve fluorescence imaging to detect the cancer biomarkers. We have studied the Human Epidermal Growth Factor 2 (HER2/neu) as one of the overexpressed cancer biomarkers in 20-30% of invasive breast cancer patients. Overexpression of HER2 receptors is one of the main factors that are involved in poor prognosis and resistance to traditional chemotherapy treatments. It is important to characterize the expression of the HER2 receptors in order to optimize the treatment procedure. To target the HER2 receptors we have used HER2-specific Affibody probe conjugated with near infrared fluorescent dye.

8940-28, Session 6

Polarization-enhanced reflectance continuous wave terahertz and optical imaging for delineating nonmelanoma skin cancers in fresh excisions

Anna N. Yaroslavsky, Rakesh Patel, Robert Giles, Cecil Joseph, Univ. of Massachusetts Lowell (United States); Victor Neel, Massachusetts General Hosptial (United States)

Background: Nonmelanoma skin cancer (NMSC) is the most common form of cancer with yearly treatment costs exceeding $600 million. Terahertz imaging has been shown to detect intrinsic differences between healthy and cancerous tissue although it suffers from low resolution and is unable to identify skin structures. Optical wide-field polarization sensitive imaging can complement terahertz imaging by providing high resolution detection of morphological features.

Materials and Methods: NMSC samples were imaged with a continuous wave (CW) terahertz system at 513 μm and wide-field polarization sensitive optical system at 440 nm. Cross-polarized terahertz images were quantified, normalized and thresholded to highlight areas suspicious for cancer. Optical imaging was used to inspect morphological appearance of the areas highlighted in terahertz images. 10 NMSC samples were imaged with CW terahertz and wide-field optical systems and the results were correlated with histology.

Results and Discussion: Pixel-by-pixel analysis revealed that quantitative terahertz imaging alone exhibited the sensitivity of 80.2% and specificity of 69.2%. Terahertz imaging was capable of identifying the gross location of cancer, but also highlighting additional areas with similar responses. Wide-field imaging was able to discern tissue morphology and discriminate benign areas within terahertz responses that were close to tumor. Together, cross-polarized CW terahertz and polarization sensitive optical imaging modalities delivered the sensitivity and specificity of 98.7% and 95.3%, respectively. This multimodal technology has the potential to offer a surgeon a quick, reliable, and comparatively low cost tool for intraoperative tumor margin delineation without the use of extrinsic contrast agents.

8940-29, Session 6

Measurement of fluorescent probes concentration ratio in the cerebrospinal fluid for early detection of Alzheimer's disease

Osnat Harbater, Israel Gannot, Tel Aviv Univ. (Israel)

The pathogenic process of Alzheimer's Disease (AD), characterized by amyloid plaques and neurofibrillary tangles in the brain, begins years before the clinical diagnosis. We propose a novel method which may detect AD up to five years earlier than current diagnosis. It is minimally invasive, with minimal risk, pain and side effects. The method is based on previous reports which relate the concentrations of biomarkers in the Cerebrospinal Fluid (CSF) (A? and Tau proteins) to the future development of AD in mild cognitive impairment patients. Our method, which uses fluorescence measurements of the relative concentrations of the CSF biomarkers, replaces the lumbar puncture process required for CSF collection.

The process uses a miniature needle coupled with an optical fiber to a laser source and a detector. The laser radiation excites fluorescent probes which were injected before and bond to the CSF biomarkers. Using the ratio between the fluorescence intensities emitted from the two biomarkers, which is correlated to their concentration ratio, the patient's risk of developing AD is estimated. A theoretical model was developed and validated using Monte Carlo simulations, demonstrating the relation between fluorescence emission and biomarker concentration. The method was tested using multi-layered tissue phantoms simulating the epidural fat, the CSF in the sub-arachnoid space and the bone. These phantoms were prepared with different scattering and absorption coefficients, thicknesses and fluorescence concentrations in order to simulate variations in human anatomy and in the needle location. The theoretical and in-vitro results are compared and the method's accuracy is discussed.

8940-30, Session 6

Third therapeutic spectral window for deep tissue imaging

Laura A. Sordillo, Sebastião Pratavieira, Yang Pu, Kaliris Salas-Ramirez, Lin Zhang, Robert R. Alfano, The City College of New York (United States)

Light at wavelengths in the red to near-infrared (NIR) region (650 nm – 2,500 nm) allows for deeper depth penetration and minimal absorption in tissue and is used in numerous medical applications. The main absorption chromophore in NIR is water. The first and the second "optical windows," which range from 650 nm to 1300 nm, were thought to be the only useful NIR wavelengths to probe tissue. Longer wavelengths above 1300 nm were ignored due to major water absorption. A new therapeutic spectral window with wavelengths between 1,650 nm and 1,840 nm is reported, which can be used for imaging deeper into tissue. In this study, the light attenuation from 300 nm to 2,500 nm, including all three optical windows is measured. Thin 100 micrometer slices from malignant and benign breast and prostate tissues and from the breast cancer cell line (MDA-MB-231) is studied. A noticeable difference between scattering coefficients of normal and malignant tissue is observed. The mean free path or total attenuation lengths (ls) in breast normal and malignant tissues and in prostate normal and malignant tissues are also obtained. A larger attenuation length can be seen in normal tissues versus malignant tissues. The attenuation length of malignant and normal tissue in the third optical window is larger than in the first and second therapeutic window. This new third near-infrared (NIR) window can provide additional information not available in the first and second therapeutic windows and may suggest a better optical imaging method for distinguishing malignant from normal tissues.

8940-31, Session 6

Spectral discrimination of blood components of thalassemia and iron deficiency anemia in adult patients

Vadivel Masilamani, Mohamad Saleh AlSalhi, S. Devanesan, King Saud Univ. (Saudi Arabia); K. Perinbam, Government Arts College Nandanam (India); M. Ravikumar, Government Arts college Nandanam (India); Siddanna Palled, Kidwai Memorial Institute of Oncology (India)

Hemoglobin is a metallo protein which transports oxygen between the organs. Thalassemia is a special type of blood disorder in which ?-chain of hemoglobin is faulty (called ?-Thalassemia). Occasionally such faults happen in ?-chain too (?-Thalassemia).

Thalassemia is generally diagnosed by blood chemistry followed by HPLC which is expensive. Iron Deficiency Anemia (IDA) is also a hemoglobinopathy which is different from thal as IDA is the result of insufficient dietary intake of iron or loss of iron through various causes of RBC. Often these two are misdiagnosed.

The present study is aimed at differentiating thalassemia patients from IDA ones based on the spectral analysis of fluorescent biological molecules in blood. For this purpose, blood samples from healthy, thalassemia and IDA individuals, each 20 in number, were collected in EDTA vials. Then centrifugation was done to get supernatant plasma and analyzed by Stoke's shift spectroscopy Similarly the acetone

extract of formed elements were subjected to the fluorescence emission spectroscopy.

The results showed that all the three sets could be classified separately with 95% accuracy. For example a very reliable discrimination factor was the intensity ratio between the NADH and flavin molecules found in plasma. This ratio is about one for normal, but 0.5 for IDA patients and 0.2 only for thal patients. The present study showed that the novel technique could be employed as promising procedure in screening.

8940-32, Session 7

Ultimate ultrafast white light's first observations: early discovery circa 1970 *(Invited Paper)*

Robert R. Alfano, The City College of New York (United States)

The first discovery and mechanism of super continuum generation with ultrashort pulses in solids (glasses and crystals) and rare gas media will be presented. How the observation of the white light over 6000cm-1 was unraveled for the first time with excitation of ultrashort pulses 45 years ago.

8940-33, Session 7

Evolution of the supercontinuum source *(Invited Paper)*

James Roy Taylor, Imperial College London (United Kingdom)

Spectral broadening and the generation of new frequencies were initially observed in pulsed laser systems in the mid-1960s as an inherent feature of the uncontrollable nonlinear process such as self-focussing and self-phase modulation occurring primarily in the gain media and were looked upon as deleterious rather than a resource. With the advent of mode locked lasers to generate picosecond pulses new effects were observed. Developed by the Alfano group in bulk media external to the laser in the 1970s the supercontinuum or "white light" source has now evolved into a commercially successful and highly compact source that can readily extend over more than three octaves with spectral power densities exceeding 100mW/nm. In this presentation I will describe this remarkable evolution.

8940-34, Session 7

Supercontinuum generation in optical fibers and its biomedical applications *(Invited Paper)*

Govind P. Agrawal, Univ. of Rochester (United States)

A microstructured optical fiber was first used in 2000 for supercontinuum generation. Since then, enormous progress has been made in understanding, controlling, and marketing fiber-based supercontinuum sources. In particular, biomedical applications of such sources are revolutionizing the field of medical imaging. In this talk I review the recent progress in this area and describe how a supercontinuum can be employed for biomedical imaging using the techniques known as coherent anti-Stokes Raman scattering, stimulated emission-depletion microscopy, and optical coherence tomography.

8940-35, Session 7

White light for the fast lane: supercontinuum generation in all-normal dispersion fibers for ultrafast photonics *(Invited Paper)*

Alexander M. Heidt, Univ. of Southampton (United Kingdom)

This talk will give an overview of the unique properties of supercontinuum generation (SCG) in all-normal dispersion (ANDi) fibers pumped by ultrashort pulses and the possibilities they offer for ultrafast photonics applications. In contrast to their anomalously pumped counterparts, the SCG process in ANDi fibers conserves a single ultrashort pulse in the time domain, completely suppresses soliton formation and decay, and avoids noise-amplifying nonlinear dynamics. The resulting spectra combine the best of both worlds – the broad, more than octave-spanning bandwidths usually associated with anomalous dispersion pumping with the high temporal coherence, pulse-to-pulse stability and well-defined temporal pulse characteristics known from the normal dispersion regime.

These characteristics are ideally suited for ultrafast photonics, and I will present application examples including the generation of high quality single-cycle pulses and their amplification, as well as ultrafast spectroscopy. This talk will also explore the exciting new possibilities enabled by extending this approach into the mid-IR spectral region using novel soft glass fiber designs.

8940-36, Session 7

Supercontinuum generation in microstructure fiber at the advent of femtosecond combs *(Invited Paper)*

Steven T. Cundiff, JILA (United States)

The development of frequency combs based on femtosecond lasers revolutionized optical frequency metrology, enable optical atomic clocks and is essential to the production of atto-second pulses.

Frequency combs are produces by locking the offset frequency of the laser, which in turn is most easily done if the spectrum spans an octave. Supercontinuum generation in microstructure fiber can easily span an octave, even for the nanojoule pulses produced by a mode-locked laser, while preserving coherence, and thus the comb spectrum.

8940-37, Session 7

Collapsing light really shines *(Invited Paper)*

Alexander L. Gaeta, Cornell Univ. (United States)

The history of super continuum generation with ultrashort pulses in bulk media will be reviewed. In particular, a description on how the self-focusing dynamics leads to shock formation and the generation of extremely broad spectra when an ultrashort pulse travels through a transparent gas, liquid, or solid.

8940-38, Session 7

Cross-phase modulation in optical Kerr media: from early discovery works to recent all-optical applications *(Invited Paper)*

Patrice L. Baldeck, Univ. Joseph Fourier (France)

Kerr cross-phase modulation (XPM) occurs when optical waves co-propagate in instantaneous intensity-dependent media. This all-optical effect leads not only to phase changes, but also to frequency, amplitude and spatial effects. In 1986, the first experiment reported the spectral broadening of a probe pulse by a pump pulse. Subsequent experiments demonstrated optically-induced phenomena, such as frequency shift, amplitude modulation, and spatial focusing that have been investigated in thousands of publications during the last two decades.

Conference 8941A: Optical Interactions with Tissue and Cells XXV

Monday - Tuesday 3 –4 February 2014

Part of Proceedings of SPIE Vol. 8941 Optical Interactions with Tissue and Cells XXV; and Terahertz for Biomedical Applications

8941-1, Session 1

Erbium laser tissue interaction: from bench to bedside *(Invited Paper)*

Martin Frenz, Univ. Bern (Switzerland)

No Abstract Available

8941-2, Session 1

Photons kill, cure, and diagnose: what's next? *(Invited Paper)*

Joseph T. Walsh Jr., Northwestern Univ. (United States)

No Abstract Available

8941-3, Session 1

From cooking egg whites to gold nano-particles: a 25 year journey *(Invited Paper)*

Massoud Motamedi, The Univ. of Texas Medical Branch (United States)

No Abstract Available

8941-4, Session 2

Enhanced imaging techniques for research and education of medical professionals: playing "Mythbuster" for 25 years *(Invited Paper)*

Rudolf M. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands); Tjeerd de Boorder, Herke Jan Noordmans, John H. G. M. Klaessens, Univ. Medical Ctr. Utrecht (Netherlands); Albert J. Van der Veen, Vrije Univ. Medical Ctr. (Netherlands)

No Abstract Available

8941-5, Session 2

Laser surgery to imaging to image-guided surgery in 25 years *(Invited Paper)*

Joseph A. Izatt, Duke Univ. (United States)

No Abstract Available

8941-6, Session 2

PDT: Back to the future (25 years of follies and fortunes) *(Invited Paper)*

Tayyaba Hasan, Massachusetts General Hospital (United States); David H. Kessel, Wayne State Univ. (United States)

No Abstract Available

8941-7, Session 2

Photofrin as a gateway drug: how PDT can lead to hardcore tissue optics and obsession with oxygen metabolism *(Invited Paper)*

Bruce J. Tromberg, Beckman Laser Institute and Medical Clinic (United States)

No Abstract Available

8941-8, Session 3

Melanin microcavitation threshold in the near infrared

Morgan S. Schmidt, Paul K. Kennedy, Air Force Research Lab. (United States); Rebecca L Vincelette, Kurt J. Schuster, Gary D. Noojin, TASC, Inc. (United States); Andrew W. Wharmby, Robert J. Thomas, Benjamin A. Rockwell, Air Force Research Lab. (United States)

In the field of laser bioeffects, the type of cellular damage induced depends on the laser wavelength and exposure duration. In the nanosecond to microsecond regime, melanin microcavitation is the damage mechanism responsible for retinal threshold lesions. In this process, melanin granules are locally heated, resulting in the formation of microcavitation bubbles. When these bubbles expand and collapse, they break the cell membrane, resulting in cell death. Melanin microcavitation has been thoroughly studied for visible and near-infrared (NIR) wavelengths for nanosecond to microsecond laser exposures. We expand this wavelength regime by exposing melanin granules to nanosecond pulses in the 1.0 to 1.3 micrometer range.

Thresholds for microcavitation of single bovine and porcine retinal melanosomes were determined using single nanosecond laser pulses in the NIR (1000 – 1319 nm) wavelength regime. Both heavy and light melanosome fractions were studied to determine if thresholds varied as a function of melanosome type. Melanosomes were irradiated using a Nd:YAG coupled with an OPO (1000 – 1200 nm) as well as a custom q-switched Nd:YAG (1319-nm laser). Time-resolved microscopy was accomplished by varying the delay between the irradiation beam and an illumination beam allowing stroboscopic imaging of microcavitation events. Results indicated an exponential increase in fluence threshold for single pulses with increasing wavelength. There were no statistical differences between bovine and porcine thresholds at the same wavelength and pulse duration. Furthermore, threshold values between heavy and light melanosomes were statistically equivalent. Additionally, absorption coefficient variation of melanosomes in the 1000 – 1319 nm wavelength regime were determined.

8941-9, Session 3

New techniques for imaging pressure waves induced by pulsed lasers

Rudolf M. Verdaasdonk, Vrije Univ. Medical Ctr. (Netherlands)

The interaction of pulsed lasers with biological tissues is usually characterized by instant vaporization of the tissue water resulting in explosive expanding and imploding vapor associated with large mechanical forces that can (un)intentionally damage tissues. A good understanding of these forces will contribute to optimizing and the safety of clinical treatments. However, the dynamics of local stress and pressure have been difficult to study.

Two new methods have been developed to visualize the mechanical stress and the dynamics of pressure waves and have been applied to study the mechanical effects around shaped fiber tips (bare, tapered and ball) coupled to pulsed lasers (Erbium 2.78 µm and Holmium 2.01 µm). The fiber tip was submerged in either beer or a slab of polyacrylamide gel. At the moment of a laser pulse, a cloud of carbon dioxide micro bubbles was formed in beer representing the area of instant pressure change inducing mechanical stress (method 1). Also the momentum of the pressure wave could be appreciated by the flow of the bubble cloud after the pulse. The effects were captured with high speed imaging at 2000 f/s. The transfer of the pressure wave after the pulse could be visualized by observing the optical deformation of a fine grid pattern observed through water or gel using digital subtraction software (method 2). The shape of the fiber tip showed to have effect on the dynamics of the pressure wave and local stresses distribution.

Carbon dioxide micro bubbles induced by local pressure change and optical techniques to detect deformation are new effective imaging techniques to study pressure waves and contribute to safe application.

8941-10, Session 3

Focusing of photomechanical waves with an optical lens for depth-targeted molecular delivery

Takuichirou Shimada, Keio Univ. (Japan); Shunichi Sato, Satoko Kawauchi, Hiroshi Ashida, National Defense Medical College (Japan); Mitsuhiro Terakawa, Keio Univ. (Japan)

We have been developing molecular delivery systems based on photomechanical waves (PMWs), which are generated by irradiation of a laser absorbing material with nanosecond laser pulses. This method enables highly site-specific delivery in the horizontal plane of the tissue. However, targeting in the vertical direction is a remaining challenge. In this study, we fabricated a novel PMW focusing device for deeper tissue targeting. A commercial optical concave lens (diameter, 25 mm) and black natural rubber sheet (laser absorber) (thickness, 0.5 mm) were attached to the top and bottom end of a cylindrical spacer (height, 10 mm), respectively, which was filled with water. A laser pulse was transmitted through the lens and water and hit the rubber sheet, generating a PMW. The PMW was propagated downward and upward. The downward wave (1st wave) was diffused, while the upward wave was reflected with the concave surface of the lens and focused at a depth determined by the geometrical parameters (2nd wave). To attenuate the 1st wave, a silicon sponge rubber disk (diameter, 2.0 mm) was adhered just under the natural rubber on the laser axis. With a lens of f = -40 mm, the 2nd wave was focused to a diameter of 6 mm at a depth of 20 mm, which was well agreed with the result of ray trace computer analysis. At a laser fluence of 5.1 J/cm2, peak pressure of a PMW reached 40 MPa at a focal point, suggesting capability of molecular delivery to deep targeted tissue.

8941-12, Session 3

Analysis of thermal effects in endoscopic nanocarriers-based photodynamic therapy applied to esophageal diseases

Irene Salas-García, Félix Fanjul-Vélez, Noé Ortega-Quijano, Univ. de Cantabria (Spain); Otakar Wilfert, Lucie Hudcová, Juraj Poliak, Peter Barcik, Brno Univ. of Technology (Czech Republic); José Luis Arce-Diego, Univ. de Cantabria (Spain)

The incidence of superficial squamous cell carcinoma in the esophagus and high grade dysplasia has steadily increased over the last decades. During this time Photodynamic Therapy (PDT) has become a promising treatment for these esophageal diseases mainly due to its noninvasive nature and the use of non ionizing radiation. However, its future viability as endoscopic mucosal treatment option requires the improvement of the present dosimetry. Emerging strategies to improve the present treatment outcome include its combination with gold nanoparticles that act as carriers for conventional photosensitizers. The inclusion of these nanoparticles in the pathological tissue could cause an increase in the optical radiation absorption. As a consequence it could lead to thermal damage in the target tissue.

In this work we propose a predictive model that allows the study of thermal effects produced when the optical radiation interacts with an esophageal disease with gold nanoparticles embedded. The model takes into account the light distribution in the tumor tissue by means of a Monte Carlo method. Mie theory is used to obtain the gold nanoparticles optical properties and the thermal model employed is based on the bio-heat equation. The complete model was applied to two types of tumoral tissue (squamous cell carcinoma and adenocarcinoma) in order to study the thermal effects induced by the inclusion of gold nanoparticles.

8941-13, Session 4

Pulse-to-pulse interaction analysis and parameter optimization for future-generation ophthalmic laser systems

Nadine Tinne, Brigitte Kaune, Sebastian Bleeker, Laser Zentrum Hannover e.V. (Germany); Holger Lubatschowski, Rowiak GmbH (Germany); Alexander Krueger, Tammo Ripken, Laser Zentrum Hannover e.V. (Germany)

We present a time-resolved photographic analysis of two or more temporal and spatial separated fs-laser pulses in water and other aqueous media. Since their product placement, a steady increase of repetition rate of the fs-laser systems used in clinical application has been established to reduce the treatment time. Therefore, the immediate pulse-to-pulse interaction becomes more and more important for future-generation ophthalmic laser systems like for the LASIK and the Femto-Phaco procedures. The basic effect of photodisruption by focusing a single ultra-short laser pulse into aqueous medium is studied in detail. As a consequence of the laser-induced optical breakdown (LIOB) an oscillating cavitation bubble appears at the focal volume, which is responsible for rupturing the tissue. By increasing the laser repetition rate this cavity will not only interact with the surrounding medium but may influence subsequent laser pulses or their cavitation bubble oscillation. For this reason, we investigated the interaction of laser pulses with different spatial and temporal separation by time-resolved photography. While there are various regimes with different characteristic interaction mechanisms, the parameter range (e.g. pulse energy, spatial and temporal separation) has been constricted regarding the medical application; here, the efficiency was optimized to a maximum mechanical impact with minimum applied pulse energy as well as unwanted side effects at the same time. In conclusion, the results of this study are of great interest for the prospective optimization of the surgical process with future-generation fs-lasers.

8941-14, Session 4

Simultaneously digital-holographic analysis during femtosecond laser-induced photodisruption in ocular tissue and material by using a pump-probe configuration

Emanuel Saerchen, Kevin Biessy, Rowiak GmbH (Germany); Björn Kemper, Univ. of Münster (Germany); Holger Lubatschowski, Rowiak GmbH (Germany)

High repetition rated femtosecond laser oscillator systems with low pulse energy are more often applied for preciser and safer eye surgery. Especially the cutting procedure in ocular lens is of great importance for presbyopia treatment. The fundamental laser tissue interaction process is not completely understood. Apparently, a self-induced interaction process takes place, were one modified region changes the focusing behavior of following laser pulses. Therefore, we used an ultra-high repeating femtosecond oscillator laser system with nJ-pulses which were focused inside the ocular-tissue-phantom material Hydroxy-ethylmethacrylat (HEMA) to cause photodisruption. The material change, caused by the fs-pulses, was measured simultaneously with a compact digital-holographic microscope. To investigate the material manipulation at different time scales, we used either a continuously illuminating light source or the femtosecond pulses itself in a pump-probe configuration for imaging of the sample. The holographic images provide quantitative values for optical path length difference (OPL), which is equivalent to a refractive index change. This change of the optical properties may cause following pulses to obtain different focusing conditions. Time lapse measurements during the laser application were performed, which show the temporal evolution of OPL. A rise of OPL during the laser application was measured, which was followed by decrease or relaxation after laser processing. Furthermore, similar experiments were performed in vitro at native ocular pig lenses. The fs-laser cutting effects in HEMA and ocular lens were transferable. Simultaneously measurements of the material modification during the cutting application give rise to better knowledge of treatment modalities during ocular lens processing.

8941-15, Session 4

Comparison of human serum and bovine serum albumins on oxidation dynamics induced by talaporfin sodium photosensitization reaction with albumin rich conditions: solution experiments

Mariko Kurotsu, Tetsuya Nakamura, Mei Takahashi, Emiyu Ogawa, Tsunenori Arai, Keio Univ. (Japan)

In order to understand extracellular-photosensitization reaction (PR) using talaporfin sodium, we studied comparison of oxidation dynamics of albumin and talaporfin sodium in solution system by visible and ultraviolet absorption spectrum measurement. Almost all talaporfin sodium particles are bound to albumin in interstitial fluid, and this binding would affect the oxidation dynamics during this PR. Bovine serum albumin (BSA) is commonly used in vitro study but its binding characteristics with talaporfin sodium are different from human serum albumin (HSA). PR was operated in a solution composed of 20 ?g/ml talaporfin sodium and 1.3 mg/ml HSA or BSA to simulate myocardial extracellular PR condition. Laser radiation of 662 nm was irradiated to this solution with irradiance of 0.29 W/cm2. Absorption spectrum of this solution was measured during the PR. We estimated oxidation ratio by absorption difference around 235 nm before and after the PR. Talaporfin sodium was oxidized 100% with HSA and BSA by the PR of 100 J/cm2 in radiant exposure. On the other hand, HSA and BSA were oxidized 60%, 94% respectively in this radiant exposure. Q-band absorption peak of talaporfin sodium with HSA was shifted to 1 nm longer wavelength increasing radiant exposure up to 100 J/cm2. This longer wavelength shift would mean binding ratio of non-oxidized talaporfin sodium to non-oxidized HSA was increased with increasing radiant exposure. Therefore it would be possible that PR with talaporfin sodium bound to HSA might present efficient PDT than PR bound to BSA.

8941-16, Session 4

Human cadaver retina model for retinal heating during corneal surgery with a femtosecond laser

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Purpose: Femtosecond lasers are widely used in everyday clinical procedures to perform minimally invasive corneal refractive surgery. The Intralase femtosecond laser (AMO Corp. Santa Ana, CA) is a common example of such a laser. In the present study a numerical simulation was developed to quantify the temperature rise in the retina during femtosecond intracorneal (Intralase) surgery. Also, transmission measurements were performed on the excised human fundus. Additionally, ex-vivo retinal heating due to IntraLase irradiation was measured with an infrared thermal camera (Fluke corp. Everett, WA) as a validation of the simulation.

Methods: A computer simulation was developed using Comsol Multiphysics and Matlab to calculate the temperature rise in the retina during Intralase surgery. Human retinas were excised from two eyes and transmission measurements with a 60kHz Intralase machine were performed. 4 human retinas were irradiated with a 150kHz Intralase and the temperature rise was measured with an infrared thermal camera.

Results: The simulation showed a temperature rise of less than 0.3 degrees for realistic pulse energies for the various repetition rates. Thermal camera measurements are in agreement with the simulation.

Conclusions: During routine Intralase surgery with normal clinical parameters, the temperature rise is well beneath the threshold for retina damage. The simulation predictions are in agreement with thermal measurements providing a level of experimental validation.

8941-37, Session PMon

Objective fitting of hemoglobin dynamics in traumatic bruises based on temperature depth profiling

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Forensic examiners are frequently faced with a need to determine the age of traumatic bruise. Currently, the age of the bruise is estimated based on subjective color. However, the perceived bruise color depends strongly on the depth of the spilled blood, natural skin tone, ambient light conditions, etc., which prevents an accurate and reliable determination of the time of the injury.

Recently, techniques such as diffuse reflectance spectroscopy (DRS) were applied to objectively quantify and analyze changes during the bruise healing process. However, DRS lacks direct information on depth distribution of chromospheres, particularly of extravasated hemoglobin, and skin geometry. Thus, some parameters have to be assumed to allow the analysis of DRS spectra. A better understanding of the underlying processes would be important for successful implementation of bruise age determination techniques.

Complementary depth information can be obtained by pulsed photothermal radiometry (PPTR). Technique allows noninvasive determination of the laser-induced temperature depth profile in strongly

scattering biological tissues including human skin. In present study, we apply this technique to characterize mass diffusion and degradation of extravasated hemoglobin during the bruise healing process. By applying Monte Carlo simulation of laser energy deposition and simulation of PPTR signal, a quantitative analysis of underlying processes is possible. We have implemented objective simultaneous fitting of a time series of PPTR data obtained during the process of bruise evolution. This allows us to derive more accurate estimates of the hemoglobin depth distribution. This technique also enables us to determine the value of hemoglobin mass diffusivity which is controversial in existing literature and has not yet been measured in vivo. In addition, individual variations of hemoglobin dynamics parameters can be quantified. Such information will be a valuable addition to existing bruise age determination techniques.

8941-38, Session PMon

Characterization of a chamber for ultraviolet irradiation of biomolecules and monitorization of structural changes by Raman spectroscopy

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The solar radiation can provide benefits for specific human cells, but can cause damage to biomolecules such as DNA, due to changes induced by the ultraviolet (UV) radiation. The photo-induced changes may be observed by the analysis of the Raman spectrum after irradiation with the desired UV wavelength (UV-A range) using a spectrometer coupled to an UV irradiation chamber. The study aimed to characterize a UV-A chamber developed in our laboratory, aiming its validation and use in studies of photo-induced changes. It has been performed measurements of the internal chamber temperature during operation, measurements of irradiation power at different sites inside the chamber, and measurement of the photodegradation of a collagen sample. The chamber is made up of MDF wood and has about 30X30X100cm, with 3 equidistant shelves. At the top of the chamber it was installed 8 UV fluorescent lamps of 8W each with emission peak between 360 and 410 nm. An UV sensor was positioned approximately 30 cm apart from the UV lamp's assembly, where it was measured about 0,1 mW/cm2 of irradiance. With room temperature of about 25°C, it was observed that the temperature was kept around 31°C at 30 cm from lamp in 30 min of irradiation. Collagen was irradiated for 60 min and it was observed changes in the Raman spectrum: decrease in the peaks of Pro/Hyp (860 - 940 cm-1), amide III (1250 - 1280 cm-1) and CH modes (1455 cm-1), indicating that the UV-A chamber was able to induce alterations in biomolecules.

8941-39, Session PMon

The effect of picosecond laser pulses on redox-dependent processes in mice red blood cells studied in vivo

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We have studied in vivo the effect of the pulsed (picosecond) laser radiation on mice red blood cells. The laser at ?=1560nm operated pulses with the pulse duration of 1,4?10-12 s , the average and peak power of 20mW and 3,7 kW, respectively.

The following peroxidation parameters of lipids exposed to the irradiation have been analyzed from the experimental data: quantitative estimations of diene conjugates, ketodienes and conjugated trienes, malondialdehyde, Schiff's base and enzyme activity of antioxidant defense- catalase, glutathione-S transferase, and superoxide dismutase in red blood cells and blood plasma of irradiated mice. The two groups of mice were irradiated at the energy dose of 3.8J/cm2. However, the animal from the first group were exposed once to the irradiation treatment at the total dose of 3.8J/cm2, while the second group was subjected to four irradiation trials getting a total energy dose of 3.8J/cm2.

The essential difference in the studied parameters has been registered for the animals from two groups. Consequently, this result could indicate a considerable effect of a pulsed (pico- and subpicosecond) laser radiation at relatively low average power on redox-dependent processes in mice red blood cells.

8941-41, Session PMon

Scattering coefficients of cancerous and normal human prostate tissues in near infrared range

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The optical coefficients of human cancerous and normal prostate tissues were investigated and compared in the near infrared spectral range. Mie theory is a powerful tool which provides analytical solution of Maxwell's equations for the photon migration in turbid media scattered by particles with size comparable with the wavelength of light in terms of scattering angle theta, wavelength ?, particle size x, and the index of refraction m of scattering particles relative to the suspension medium.

In this study, spectral measurements and Mie theory were utilized to study the optical properties of ex vivo normal and cancerous prostate tissues. The studies were carried out for both tissue and cell suspensions. Using the experimental results and the parameters of the refractive index of cytoplasm, size of nuclei, and the diameter of the nucleoli for cancerous and normal human prostate tissue reported from the biological and biomedical studies, we report our initial results how these parameters vary between these two types of tissues. Wavelength dependence of near infrared light absorption and scattering can be used to reveal structures of tissue and cells. Preliminary results show that normal and malignant prostate tissues can be discriminated from the difference of their optical coefficients. This study serves as a critical step to establish connection between the characteristics of light scattering to the complex structure of tissue and cells.

8941-42, Session PMon

Optimization of the interstitial PDT light dosimetry

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Photodynamic therapy (PDT) has been used for treatment of several tumor types, and presents best results for surface lesion. Light penetration on biological tissue is one limiting factor in PDT and cylindrical diffusers have been used for the application interstitial in solid and larger tumors. However, differences in the diffuser light emission depend on the manufacturing process, size and optical properties of the fiber, which make it difficult to establish light dosimeter. This study aims to determine the distribution of light generated by a cylindrical diffuser in a turbid medium. A solution of lipid emulsion was used as an optical phantom. An optical fiber with a cylindrical diffuser of 2 cm in length was connected to a diode laser 630 nm, and the spatial distribution of light generated by the diffuser was measured by scanning a collector

optical fiber. From the measurement of the light field generated by an element (1 mm long) of a 20 mm-long cylindrical diffuser, recovery of the distribution of light generated by the entire diffuser is expected. Then, the PDT was done in rat liver to analyze a real response and, with help of computational tools, a necrosis generated by irradiation of all fiber was reconstructed, using a necrosis produced by an element 2 mm long (likewise the element 1mm long). The results showed that knowing the illumination profile of a cylindrical diffuser and the light distribution in turbid medium, it was possible to redefine a shape of necrosis from as animal model theoretically.

8941-43, Session PMon

Direct and diffuse light propagation through coral tissue

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Light propagation in coral tissues is basically unstudied, although the interaction of tissue with light is central to our understanding of coral photophysiology. Here, we present insight into light propagation of coral tissue. We measured the lateral attenuation of 636 nm and 785 nm scalar irradiance with microfibres on a live coral and the on the underlying bare skeleton. Based on these measurements we develop a 2-layer Monte Carlo simulation to determine the optical properties of tissue and skeleton. Our results show intense scattering of the coral tissue, which leads to efficient light capture within the tissue layer. The underlying skeleton allows light to diffuse laterally and illuminate distant areas. We conclude that coral tissue and skeleton optics work in concert to optimise the illumination of the live tissue layer and are thus likely key factors for the observed high photosynthetic efficiency in corals.

8941-44, Session PMon

Identification of optimal wavelengths to improve broadband optical thermometry

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OBJECTIVES:

We search for optimum TR wavelengths in order to calibrate broadband continuous absorption spectrum. We investigated cross-talk for different wavelength measurements, and analyze it over various chromophore combinations and different components of pure water absorption spectra at different temperature measured from principal component analysis (PCA) [1].

METHODS:

The choice of wavelengths is based on two consideration; separation of absorption from scattering and differentiation of individual chromophores which is introduced by Corlu et al.

Hollis et al. also described the calibration of the pure water absorption spectrum against temperature as a combination of components, known as principal components (PCs), that could be used for fitting of tissue temperature [1]. A MATLAB program was written to determine these PCs.

RESULTS:

Results indicates that an optimal wavelength for pairing with 800 and 830nm for the triple-wavelength measurement of oxy- and deoxyhemoglobin (HbO2, Hb) is 760nm. When the absorption chromophores are water, HbO2 and Hb, the optimum wavelength formulation gives an optimum set with five wavelength i.e., (760, 800, 830, 870 and 920nm). Similar analysis with two principal components (PC1,2) for pure water suggest that the forth wavelength in addition to 760, 800 and 830nm should be selected from 930-970nm to minimize cross-talk between PC1,2.

CONCLUSION:

We have investigated the optimum wavelengths for different absorption chromophores and wavelength combinations to improve temperature prediction.

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8941-45, Session PMon

Experimental validation of Monte Carlo simulators of light transport through tissue phantoms

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For light based medical treatments to be effective, it is important that the correct dose of light is delivered to the treatment site. The optical properties of tissue determine the degree of scattering and absorption of light and hence the fluence at a given depth. Computer simulations can be used to predict the fluence at the treatment site, and are an invaluable tool in determining the correct dose to be delivered in therapeutic applications. In this work, reflection and transmission measurements of a tissue phantom are made using a double integrating sphere system. The optical properties are obtained by iterating an adding-doubling solution of the radiative transport equation until the calculated values of the reflection and transmission match the measured ones. The calculated optical properties were then used as input for two Monte Carlo simulators (MMC and MCxyz) and the transmission and reflection fractions were compared with the experimental results. The results showed a very good agreement with the measured values, and the simulators can therefore be used with confidence to predict fluence at the treatment site.

8941-46, Session PMon

Opto-thermal interaction of porcine stomach tissue with 808-nm laser in endoscopic submucosal dissection

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In endoscopic submucosal dissection (ESD), a difficulty accompanies the surgical interventions in the narrow gastrointestinal space. Tissue ablation apparatuses with a high-power CO2 laser or a Nd:YAG laser for use in the endoscope have been developed to facilitate the surgical procedure. We conducted a fundamental study on laser-tissue interaction with the aim of developing a therapeutic medical device that can remove lesions on the intestinal wall by irradiating an 808-nm near-infrared laser light incorporated in an endoscopic system. The perforation depth at the porcine fillet tissue was linearly increased in the range of 1~4 mm in proportional to laser output (3~12 W) and irradiation time (5~20 s).

The perforation depth of the porcine intestinal wall was 2 times smaller than the fillet tissue at the same condition. Measured temperature distribution suggests that the energy is localized in the irradiation spot and transferred to deep tissue, protecting the normal tissue from thermal injury. To further enhance the localization of the energy conversion, we investigated the opto-thermal properties, such as absorption coefficient, thermal conductivity, and specific heat, of a gel as a submucosal fluid cushion (SFC) substitute. Measurements of the perforation depth and the temperature distribution with the tunable gel indicated that the laser-tissue interaction can be controlled by tuning the opto-thermal properties of the SFC substitute, which leads to minimization of the thermal damage to the surrounding normal tissue. Our results can contribute to optimization of the driving parameters for the laser incision technique as an alternative to conventional surgical interventions.

8941-47, Session PMon

Application of the 1940-nm thulium fiber laser in stereotaxic surgery

Burcu Tunç, Tuba Akgul, Murat Gülsoy, Bogaziçi Üniv. (Turkey)

Background: Infrared fiber lasers with higher local absorption peak of water are expected to be an effective tool for photothermal laser surgery with minimal thermal damage to subjacent healthy tissue. Objectives: In this study it is aimed to show the efficacy of the 1940-nm thulium fiber laser on the subcortical tissue. The continuous-wave 1940-nm laser delivered via silica fibers would have great potential for stereotaxic neurosurgery due to its strong absorption by water. Materials and Methods: An in vivo stereotaxic surgery was performed on randomly selected, 3-3.5 months old, weighing 250-290 g 16 Male Wistar rats. After the placement of anesthetized rats to the stereotaxic frame, craniotomy was performed. A wide window was opened to expose laser light in both hemispheres. Dura was removed. Laser was then delivered for specific power settings and time durations (400 and 600 mW, 2.5-5 seconds). All subjects were sacrificed after the laser surgery. The brain samples immediately placed in 10% formalin for histological analysis. Tissue samples fixed in formalin were stained with cresyl fast violet for light microscopy and lesion zones were characterized, measured and statistically compared with ANOVA and Tukey tests. Results: Histological analysis of brain samples showed that the 1940-nm thulium fiber laser has a remarkable ability to vaporize and/or coagulate the tissue with minimal thermal damage of nearby tissue. It is found that the 1940-nm thulium fiber laser can be a promising tool for neurosurgical applications.

8941-48, Session PMon

The photothermal effects of 1940-nm thulium fiber laser on cortical tissue: in vivo dosimetry study

Burcu Tunç, Tuba Akgül, Murat Gülsoy, Bogaziçi Üniv. (Turkey)

Background: The main mechanism behind phototermal interactions is absorption and scattering of photons by the macromolecules. Biological tissues are mainly composed of water, for cortical tissue this value is 82%. Hence, 2?m lasers have a great advantage for photothermal applications due to their capability of transmitting light via silica fibers with highest absorptional peaks for water. Objective: The aim of this study is to show the efficiency of 1940-nm thulium fiber laser in removing of cortical tissue stereotaxic surgery.

Materials and Methods: Data was acquired from 16 male Wistar rats (3-3.5 months old, weighing 250-290 g). After the placement of anesthetized rats to the stereotaxic frame, craniotomy was performed. Bilateral lesions were created at primary motor cortex for the following coordinates: +3.72 mm from bregma, ±3.00 mm from the midline and, -1.12 mm below the dura. Laser was then delivered for specific power settings and time durations (400 and 600 mW, 2.5-5 seconds) and the temperature increase for the nearby tissue was monitored. After the sacrifice, the brain samples were immediately placed in 10% formalin and stained with cresyl fast violet for neuron counting and laser lesion characterization under light microscopy. Data were analyzed with ANOVA and Tukey tests. Results: A strong correlation between the number of neurons and changes in the temperature was found. Histological analysis indicated that 1940-nm thulium fiber laser could be a promising tool for cortical tissue surgery.

8941-50, Session PMon

Laser-induced fluorescence spectroscopy in tissue local necrosis detection

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Local blockage of blood vessels supplying the tissue of the small intestine is a life-threatening disease that affects patients in middle and old age. Local tissue necrosis of the intestinal tissue is an immediate consequence of this blockage of blood vessels. Immediate surgical intervention is then the only solution that can avert subsequent fatal intestinal perforation followed by some toxications of a patient body. Only immediate surgical operation allows the removal of necrotic tissue. The surgeon must quickly separate the tissue that is affected by tissue necrosis from the healthy one. On the border between necrotic and healthy tissue is necessary to keep the surgical cut. Therefore special technique of that assessment is needed. Fluorescence spectroscopy as a nondestructive technique allows online in vivo analysis of organics and biological materials. The relation between excitation wavelength and obtained emission spectrum is given by molecular structure of the investigated sample and it makes it unique for each material. Laser-induced differential fluorescence spectroscopy is a technique taking advantage of the fluorescence ability to provide discrimination between different materials or organic structures. In medical research it is experimentally used e.g. for identifying of a pathological tissue without biopsy. The presented work scopes to a pilot experiment using pulse UV laser and the differential laser induced fluorescence spectroscopy technique in order to detect tissue local necrosis in small intestine surgery. The designed experimental setup is based on UV pulsed laser operated at the wavelength 355 nm exiting the tissue molecular structure for evaluation of an oxygenation level. Thanks to a low contrast of snap shots obtained during this pilot experiment we applied a contrast dye - IndoCyanine Green (ICG) which absorbs and emits wavelengths in the near IR. We used our narrow-linewidth extended cavity diode laser (ECDL) for stimulating of some critical areas of the small intestine tissue with injected ICG dye. The ECDL laser works at 792 nm wavelength and we developed the laser for general spectroscopy purposes. The first file of shots of healthy and necrotic parts of the small intestine tissue was captured by Andor iXon3 high sensitivity fluorescent cam. The proof-of-concept methodology based on pulse UV laser driven differential fluorescence spectroscopy and the spectroscopy with ICG dye were demonstrated for tissue necrosis assignment. The schematic diagram, optical setup and these first results are presented in the work

8941-17, Session 5

Consolidated numerical temperature/ pressure modelling to assess the accuracy of optoacoustic temperature determination during retinal photocoagulation

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Retinal photocoagulation is an established treatment for various retinal diseases. The temperature development during a treatment can be monitored using the photoacoustic effect by applying short laser pulses in addition to the treatment laser light. The laser pulses induce thermoelastic pressure waves with a temperature dependent amplitude which represents the average voume temperature of the irradiated volume.

We present a numerical model of the background of the eye to examine the temperature development during the treatment as well as the formation, propagation and detection of the ultrasonic waves. Using the model, it is possible to investigate the accuracy of the temperature determination under varying conditions such as inhomogeneous pigmentation or change of the irradiation parameters, such as the laser light beam shape.

It was shown that there is an uncertainty of up to 9% in the determination of the peak temperature when the absorption coefficient between the absorbing layers is varied by a factor of up to 2.

Furthermore, for an excitation pulse duration of 75 ns, the resulting pressure wave energy is attenuated by 76 % on its way from the retina to the cornea due to frequency dependent attenuation in water. A Fourier analysis of the emitted pressure waves was performed to quantify the influence of the beam shape on the wave amplitude and frequencies when changing the beam diameter and changing the beam from a top hat to a Gaussian profile.

8941-18, Session 5

Observation of changes in membrane fluidity after infrared laser stimulation using a polarity-sensitive fluorescent probe

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It has been shown that exposure of live neurons to a low-intensity pulsed infrared light can be used to excite action potentials. Infrared pulsed laser coupled to an optical fiber can be utilized to create a rapid localized increase in temperature in the vicinity of the cell. The resulting temperature gradient leads to an increase in membrane fluidity and permeability, causing depolarization of the target cell. In order to characterize the fluidity of the cell membrane at various temperatures with and without pulsed IR light exposure, we used a polarity-sensitive fluorescent probe di-4-ANEPPDHQ. This dye exhibits a fluorescent shift between the disordered and ordered phases of the membrane, and can be used to quantitatively evaluate the state of the membrane by calculating the generalized polarization (GP) value. Using high-speed imaging of cells exposed to a pulsed IR light of varying pulse width it was determined that a longer pulse width leads to a greater change in the GP value. Comparison of GP values of cells at different ambient temperatures without the pulsed IR light exposure and cells exposed to pulsed IR light indicated that a rapid temperature gradient caused by the exposure to pulsed light induces a larger change in GP value, indicating a greater disruption of membrane fluidity and permeability.

8941-19, Session 5

Thermal and damage data from multiple microsecond pulse trains at 532 nm in an in vitro retinal model

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Using an established artificially pigmented in vitro retinal model we determined laser damage thresholds at 532 nm for 1, 10, 100, and 1000 pulses (~230-µs/pulse at 50 pulses/s). Full-frame thermal images were recorded during laser exposure with effective pixel dimensions of 8 x 8 µm (microthermography), and necrotic death was determined post-exposure using fluorescent indicator dyes. In order to induce damage from a single laser pulse, we had to increase intracellular pigmentation to levels not previously reported and use a small beam diameter (130 µm). Radiant exposure estimated dose values expected to generate damage 50% of the time (ED50) followed expected trends and all had good 95% confidence intervals. The reduction of ED50 values as a function of number of pulses followed a power function with an exponent of (-0.121). By increasing intracellular pigmentation by 50% the power function had an exponent of (-0.061). Thermal data was complicated by the difference between frame rate and laser pulse duration but was useful in computational models, especially when using thermal profiles from 2-s CW exposures as a reference for the 100 pulse exposures.

8941-20, Session 5

Semi-dynamical cryo-imaging study of laser-tissue vaporization and coagulation process

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Laser surgery has been popularly adapted in many clinics. The concepts of coagulation and vaporization have been generally used to explain the physical mechanisms of laser-tissue interaction for laser surgery. However, the relation between these two dynamic process mechanisms during laser cutting nor the associated parameters such as temperature variation have not been observed, quantified, or thoroughly understood. In this presentation, we try to provide new insight into tissue coagulation and vaporization by using a cryo-imaging method to dynamically observe and replay the processes. In addition, we also develop a method to monitor the relative temperature variation during the vaporization and coagulation in real-time. By combining both methods, we are able to identify the tissue vaporization thresholds under different power densities and disclose how the coagulation depth is related to these thresholds. Furthermore, we quantified the vaporization area and coagulation depth and identified the dominant factors affecting both parameters. The results will point the way to developing new lasers and controlling mechanisms for effective tissue vaporization, better hemostasis, and for reducing potential complications, such as dysuria, during or after laser surgery.

8941-21, Session 5

Acute cell death rate of vascular smooth muscle cells during or after short heating up to 20 s ranging 50 to 60? as a basic study of thermal angioplasty

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We studied the relations between the time history of smooth muscle cell (SMC) death rate and heating condition in vitro to clarify cell death mechanism in heating angioplasty, in particular under the condition in which intimal hyperthermia growth was prevented in vivo swine experiment.

A flow heating system on the microscope stage was used for the cell death rate measurement during or after the heating. The cells were loaded step-heating by heated flow using a heater equipped in a Photo-thermo dynamic balloon. The heating temperature was set to 37, 50-60?. The cell death rate was calculated by a division of PI stained cell number by Hoechst33342 stained cell number.

The cell death rate increased 5-10% linearly during 20 s with the heating. The cell death rate increased with duration up to 15 min from 5 s heating. Because fragmented nuclei were observed from approximately 5 min after the heating, we considered that the cell death mechanisms were come from acute necrosis and late necrosis, within 5 min and over 5 min, respectively. Late necrosis is probably corresponding to apoptosis. The ratio between the acute and late necrosis were calculated based on this consideration as 1.4:1 under the particular condition in which intimal hyperthermia growth was prevented in vivo previous swine experiment.

We think that this ratio of necrotic interaction is larger than that expected to obtain intimal hyperplasia suppression.

8941-22, Session 6

Reconstruction of double tumors in vivo based on the fluorescence molecular tomography

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Cancer threatens human's health with a long time, and the primary tumors are found in more than one position constantly, so it is important that diagnose and reconstruct multiple lesions accurately. Among molecular imaging, fluorescence molecular tomography (FMT) is a promising technique that can three-dimensionally resolve the molecular processes by localizing the fluorescent probes. In recent years, an increasing effort has been devoted to the optimization of acquisition and reconstruction schemes for FMT. Consequently, the precision of the algorithm and the efficiency of the reconstruction have been improved greatly and most of the proposed algorithms were evaluated by numerical simulations and physical experiments. However, the tumor in situ would be affected by several biotic factors. In addition, the use of FMT for preclinical studies has been limited to detection of single tumor. For that, building multiple tumors model for verifying the algorithm is very necessary. In our research, considering the location of tumors would be in different depth, we built a double tumors mouse model includes one subcutaneous tumor and one liver tumor. We choose iteratively reweighted regularization method to reconstruct the double tumors. After that, we cut the subcutaneous tumor out and utilize the same method to reconstruct liver tumor alone. We compared the two reconstruction results with the mouse structure diagram, and found our reconstruction results were in accord with the position of real tumors.

8941-23, Session 6

Optically clearing tissue as an initial step for 3D imaging of core biopsies to diagnose pancreatic cancer

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Since the late 1930s, pathology has consisted of fine needle aspirations for the diagnoses of various diseases. In the early 1990s, advancements led to the current procuring of core biopsies (CBs), which consists of obtaining tissue cores (L = 1-2 cm, D = 0.4-2.0 mm) using a needle and observing CBs using bright-field microscopy. However, between procurement and visualization, biospecimens must be processed, sectioned and mounted on flat slides for 2D visualization. Consequently, an increasing proportion of optical information about the native tissue state is lost with each procedural step. This is further confounded by a pathologist's interpretation of the 3D tissue distribution in a CB by observing sequential slides containing sections 1-8 µm in thickness. Therefore, how would pathology and disease assessment improve if entire, intact CBs were imaged in both bright-field and 3D? CBs are mechanically delicate, therefore a simple device was made to cut intact, simulated CBs (L = 1-2 cm, D = 400-500 µm) from pig pancreas. After CBs were laid flat in a chamber, z-stack images at 40 and 20x were acquired through the sample with and without the application of an optical clearing agent (FocusClear®). Intensity of transmitted light increased on average by 10x and islet structures unique to pancreas were clearly visualized 250-300 µm beneath the tissue surface. CBs were then placed in index matching square capillary tubes and bright-field z-stack images were acquired at 0°, 90°, 180° and 270° to present 3D visualization of the entire CB to the pathologist.

8941-24, Session 6

Effect of an integrating sphere measurement on the distortion of a laser pulse propagating through a turbid medium

Beatriz Morales Cruzado, Francisco G. Perez-Gutierrez, Dirk Frederik De Lange, Ricardo Romero-Méndez, Univ. Autónoma de San Luis Potosí (Mexico)

A focused nanosecond laser pulse produces optical damage to subsurface targets when its intensity is high enough to overcome the required threshold irradiance. However, when the material is highly scattering, the laser pulse irradiance decreases as it propagates through the sample because the pulse's temporal profile is stretched due to multiple scattering events. The objective of this work is to determine how much stretching a nanosecond laser pulse suffers as it propagates through a turbid medium as a function of the sample's scattering coefficient considering the integrating sphere effect. Integrating spheres are used to measure the total diffuse reflectance and transmittance of homogeneous turbid media samples to retrieve its absorption and scattering coefficients. Reflectance and transmittance measurements, being static properties, are not affected by multiple reflections of light inside the integrating spheres. However, for a time-dependent measurement, such as the temporal profile of a short laser pulse propagating through a turbid medium, light reflected and scattered multiple times inside the sphere deforms the pulse's temporal profile measured, which complicates its measurement. In this work we present results of the effect caused in the intensity profile of a nanosecond laser pulse propagating through turbid media using integrating spheres. From our results, we were able to extract the temporal transfer function of the integrating sphere, and remove this effect from the signal measured to get the true intensity profile, resulting from the interaction of the laser

pulse with the turbid sample. We compare our experimental results with Monte Carlo simulations.

8941-25, Session 6

Adaptive focus for deep tissue using diffuse backscatter

Jeremy Kress, Kambiz Pourrezaei, Drexel Univ. (United States)

A system integrating high density diffuse optical imaging with adaptive optics using MEMS for deep tissue interaction is presented. In this system, a laser source is scanned over a high density fiber bundle using Digital Micromirror Device (DMD) and channeled to a tissue phantom. Backscatter is then collected from the tissue phantom by a high density fiber array of different fiber type and channeled to CMOS sensor for image acquisition. Intensity focus is directly verified using a second CMOS sensor which measures intensity transmitted though the tissue phantom. A set of training patterns are displayed on the DMD and backscatter is numerically fit to the transmission intensity. After the training patterns are displayed, adaptive focus is performed using only the backscatter and fitting functions. Additionally, tissue reconstruction and prediction of interference focusing by photoacoustic and optical tomographic methods are discussed. Finally, potential NIR applications such as in-vivo adaptive neural photostimulation and cancer targeting are discussed.

8941-51, Session Key

Overview of the military medical photonics program

Howard Schlossberg, Air Force Office of Scientific Research (United States)

No Abstract Available

8941-26, Session 7

Development of a simulation toolbox for predicting light distribution in rat brain tissue during optical stimulation

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Having a good estimation of optical properties of tissue is helpful in light-tissue interaction applications. Optogenetics is an advanced method which combines optics and genetic techniques for brain stimulation. Delivering the correct amount of light for stimulation of the target area in the brain is an important step in optogenetic neural stimulation; an insufficient amount of optical power cannot stimulate the target area while excessive power might damage the tissue or stimulate unintended areas in the brain. Therefore, predicting the distribution of light in the brain tissue under different light delivery conditions is valuable for optogenetic research.

The process of extracting the optical properties of the rat brain tissue requires three measurements during laser irradiation: the amount of light transmitted through the tissue, the amount of light reflected from the tissue, and the amount of ballistic transmission through the tissue. These are measured using two integrating spheres and a telecentric lens with brain slices at thicknesses of 500 micrometers. Each slice is scanned with a spatial resolution of about 800 to 1200 points for three different wavelengths: 445nm, 532nm, and 635nm. We use the Inverse Adding-Doubling (IAD) technique to reconstruct the optical properties including absorption, scattering and anisotropy coefficients, from measured values. Using these estimated values, we developed a 3D database of the optical properties of the brain. Next, a Monte Carlo toolbox was added to use the values stored in the database and simulate the light-tissue interaction in the visible spectrum.

8941-27, Session 7

Theory and experiments on time-resolved reflectance from adult heads for functional tomographic imaging of brain activities

Tadatoshi Tanifuji, Masahiro Suzuki, Kitami Institute of Technology (Japan)

The finite difference time domain (FDTD) analysis has been formulated for predicting time-resolved reflectance from a realistic adult head model with brain grooves containing non-scattering (clear) layer. The analysis is based on hybrid transport - diffusion theory, in which the additional equivalent source functions due to the non-diffusive light across a clear region have been introduced into the diffusion equations. Mean delay (MD) and time-resolved reflectance calculated by the FDTD analysis were compared with in vivo experiments of human foreheads measured by a system composed of time-correlated single photon counting detection and a diode laser operating at the wavelength of 0.78 ?m, whose instrument response function has a FWHM of approximately 300 ps. Experimental MD dependences on source-detector separation (d) have shown quite different characteristics among three volunteers for d larger than 30mm. The numerical results based on the FDTD analysis revealed that the different characteristics arise from the different contribution of the equivalent source owing to their proper head curvature and it has been shown that MD dependences on d are predicted by the FDTD analysis. Theoretical and experimental time-resolved reflectance were also compared assuming typical optical parameters. Both the rising and falling edges of the experimental time-resolved reflectance to 1% of the maximum amplitude are shown to be predicted by the FDTD analysis. These results have shown that the tomographic imaging of brain activities is promising, which improve depth sensitivities by enhancing the contribution of late photons in time-resolved systems.

8941-28, Session 7

Absorption, thermal diffusion and specular reflection, Monte Carlo-based study on human skin using a multi-wavelength pulsed fiber laser for acupuncture applications

Solange I. Rivera-Manrique, Ctr. de Investigación e Innovación Tecnológica (Mexico); Steven L. Jacques, Oregon Health & Science Univ. (United States); José A. Alvarez-Chávez, Ctr. de Investigación e Innovación Tecnológica (Mexico)

Laser radiation using near-infrared wavelengths may improve the therapeutic window for laser acupuncture applications that have risen from traditional Chinese medicine. One of the most important parameters to be determined for the study of skin-laser light interaction is the beams radiated power per unit area, since that would set the skin damage threshold for such beam. Furthermore, laser wavelength sets, under heat diffusion processes, the penetration depth unto the skin. In order to determine the required laser parameters for this work, tissue optical properties as well as light transport phenomena were simulated using the well-known Monte Carlo process. The main objective of this work is to

provide simulation results from absorption, thermal diffusion and specular reflection simulations on light-dark to dark skin, typical in Hispanic population, in order to avoid thermal damage and other skin problems. Furthermore, the design of a multi-wavelength, pulsed, RE-doped fibre laser device for applications in acupuncture will be thoroughly described in the presentation

8941-29, Session 8

Photoinduced structural changes to protein kinase A

Sarah C. Rozinek, Lorenzo Brancaleon, The Univ. of Texas at San Antonio (United States)

The importance of porphyrins in organisms is underscored by the ubiquitous biological and biochemical functions that are mediated by these compounds and by their potential biomedical and biotechnological applications. Protoporphyrin IX (PPIX) is the precursor to heme and has biomedical applications such as its use as a photosensitizer in phototherapy and photodetection of cancer. Among other applications, our group has demonstrated that low-irradiance exposure to laser irradiation of PPIX, Fe-PPIX, or meso-tetrakis (4-sulfonatophenyl) porphyrin (TSPP) non-covalently docked to a protein causes conformational changes in the polypeptide. Such approach can have remarkable consequences in the study of protein structure/function relationship and can be used to prompt non-native protein properties. Therefore we have investigated protein kinase A (PKA), a more cancer treatment oriented protein model than our previous system. PKA's enzymatic functions are regulated by the presence of cyclic adenosine monophosphate for intracellular signal transduction involved in regulating glycogen, sugar, and lipid metabolism inside adipocytes, both skeletal and cardiac myocytes, neurons, hepatocytes, and various kidney cells. In nucleus accumbens neurons, PKA helps translate the dopamine signal into cells, and in adipocytes and hepatocytes, PKA phosphorylates metabolic enzymes. Since phosphorylation is a necessary step in some cancers and inflammatory diseases, inhibiting the protein kinases, and therefore phosphorylation, can treat these diseases. Fluorescence lifetime and circular dichroism measurements indicate small secondary and tertiary structural changes to PKA occur upon irradiation when bound to either PPIX or TSPP at physiological pH. These photoinduced structural changes could affect the protein's enzymatic and signaling capabilities.

8941-30, Session 8

Immediate response of Ca2+ concentration in myocardial cells against oxidation stress by extracellular photosensitization reaction using Talaporfin sodium for the arrhythmia treatment application

Emiyu Ogawa, Mei Takahashi, Arisa Ito, Tsunenori Arai, Keio Univ. (Japan)

We studied the immediate response of myocardial cells by continuous observation using confocal microscope against oxidation stress by extracellular photosensitization reaction using Talaporfin sodium for tachyarrhythmia treatment application. Immediate response in order from several seconds to several minutes is required for the arrhythmia treatment since operators should judge the therapeutic effect during the tachyarrhythmia ablation procedure. To understand the immediate response of myocardial cells, we measured the intracellular Ca2+ concentration using fluo-4 AM during and after the extracellular photosensitization reaction. Talaporfin sodium concentration was varied 10-30 ?g/ml. A red diode laser of 663 nm in wavelength was irradiated under the microscope with the radiant exposure of 10-40 J/cm2 and irradiance of 0.29 W/cm2. We observed the fluorescence image of fluo-4 AM each 400 ms during and 10 min after the photosensitization reaction. The myocardial cells beating was stopped about 2 s after the beginning of the laser irradiation. The blebs were formed with the Ca2+ inflow. The intracellular Ca2+ was re-decreased after the bleb formation and then the cell necrosis was induced. The cell lethality 10 min after the laser irradiation was less than bleb formation ratio. The time response of the cell necrosis was shortened with the photosensitizer concentration increasing and the minimum average value was 209 s in the case of the 30 ?g/ml in photosensitizer concentration and 40 J/cm2 in the radiant exposure. We think this extracellular photosensitization reaction is applicable to arrhythmia treatment in terms of its immediate response.

8941-31, Session 8

Photosensitization reaction along depth of a culture well with high concentration of talaporfin sodium for extra-cellular photodynamic therapy study

Masahiro Yajima, Hiroshige Kawakami, Emiyu Ogawa, Mei Takahashi, Tsunenori Arai, Keio Univ. (Japan)

We studied photosensitization reaction progress in the well commonly used in cell culture/interaction study by oxygen partial pressure distribution measurement with a high concentration of talaporfin sodium to simulate extra-cellular photodynamic therapy. The talaporfin sodium solution of 20 ?g/ml in concentration with 2.8 mm thickness in the well was irradiated up by 663 nm excitation laser with 0.29 W/cm2. A Clark-type oxygen electrode was used to measure oxygen partial pressure during the photosensitization reaction, with the correlation of the solution temperature and laser irradiance. The oxygen partial pressures in any measuring points ranging 0.5 to 2.0 mm in depth of the solution during the photosensitization reaction were decreased up to 0.7 J/cm2 radiant exposure. Then these pressures decreased very slowly up to 40 J/cm2. There are significant difference in oxygen partial pressure gradient between 0.5 mm and 1.0 mm in depth comparing to that between 1.0 mm and 1.5 mm after 40 J/cm2 radiant exposure. We also found that talaporfin sodium absorption in shallow layer (0.5 mm) was thicker than that in deep layer (1.0 mm). Therefore, we revealed that huge distribution on talaporfin sodium concentration along depth in the well with above mentioned situation which was simulated as the extra-cellular photosensitization reaction was existed, that is, the photosensitization reaction progress in the shallow layer in the well was faster than that in deep layer in the well under high radiant exposure.

8941-49, Session 8

Laser based intracellular drug delivery with endocytozed peptide-conjugated gold nanoparticles

Judith Krawinkel, Friedrich-Schiller-Univ. (Germany); U. Richter, M. L. Torres-Mapa, B. Tumursukh, Friedrich-Schiller-Univ. Jena (Germany); Lisa Gamrad, Christoph Rehbock, Univ. Duisburg-Essen (Germany); H. Murua Escobar, Univ. Rostock (Germany); Anaclet Ngezahayo, Leibniz Univ. Hannover (Germany); Stephan Barcikowski, Univ. Duisburg-Essen (Germany); Alexander Heisterkamp, Friedrich-Schiller-Univ. Jena (Germany)

Recent studies have shown that gold nanoparticles for laser based cell manipulation are effectively utilized to deliver molecular compounds into living mammalian cells. Pulsed laser irradiation of gold nanoparticles attached to the cell membrane can induce transient perforations and allow the delivery of exogenous molecules into the cell. However, successful molecular delivery critically depends on size and number of pores created on the membrane.

To overcome the necessity of rupturing the cell membrane and to enable local subcellular drug delivery, we explore the use of peptide-conjugated gold-nanoparticles (PCGNs) fabricated by laser ablation. In this work, these particles were conjugated with CWR10 to facilitate the particles entry into cells via endocytosis. Co-Incubation of PCGNs with molecules such as Calcein or larger FITC-Dextrans permit the intracellular uptake of both. To release these intraendosomal contents into the cytoplasm, plasmonic effects are induced by irradiation of a pulsed laser (532 nm, 1 ns), thereby producing local disruption on the endosomal membrane without damaging the outer cell membrane. The release is observed by the diffusion of the fluorescently-labeled molecules into the cytoplasm. Incubation of cells with a nucleic acid stain during irradiation shows that cell membrane integrity is maintained during the process. Furthermore we monitor no significant decrease in cell viability 24 h after irradiation. Therefore, our technique can be an enabling method for high throughput delivery of molecules, such as siRNA or larger molecules, into mammalian cells.

8941-32, Session 9

The optical properties of whole blood: a critical review and theoretical approach

Nienke Bosschaart, Gerda J. Edelman, Maurice C. G. Aalders, Ton G. van Leeuwen, Dirk J. Faber, Univ. van Amsterdam (Netherlands)

Optical property measurements on blood are influenced by a large variety of factors of both physical and methodological origin. The aim of this study is to review and list these factors of influence, and to provide optical property spectra (250 – 2500 nm) for whole blood that can be used in the practice of biomedical optics. Hereto, we perform a critical examination and selection on the available optical property spectra of (whole) blood in literature, from which we compile average spectra for the absorption coefficient (μa), scattering coefficient (μs) and scattering anisotropy (g). From these compiled spectra, we calculate the reduced scattering coefficient (μs') and the effective attenuation coefficient (μeff). Furthermore, we provide practical tools for extrapolating μs to other wavelengths and rescaling μs to other hematocrit levels.

Since the measurement of the scattering properties of blood has proven to be challenging, we apply an alternative, theoretical approach to calculate spectra for μs and g. Hereto, we combine Kramers-Kronig analysis with analytical scattering theory, extended with Percus-Yevick structure factors that take into account the spatial correlations between the positions of individual red blood cells in a whole blood medium. We argue that our theoretical spectra may provide a better estimation for μs and g (and hence μs' and μeff), than the compiled spectra from literature.

8941-33, Session 9

Absorption spectroscopy of melanin in fresh tissue sections from pigmented lesions

Barukh Rohde, Israel Coats, James Krueger, Daniel S. Gareau, The Rockefeller Univ. (United States)

The optical properties of pigmented lesions have been studied using diffuse reflectance spectroscopy in a noninvasive configuration on optically thick samples such as skin in vivo. However, it is difficult to un-mix the effects of absorption and scattering with diffuse reflectance spectroscopy techniques due to the complex anatomical distributions of absorbing and scattering biomolecules. We present a device and technique that enables absorption measurements of tissue volumes much smaller than the optical mean-free path. Our results on lesions from 20 patients including melanomas and nevi show the absorption spectrum of melanin in melanocytes and basal keratinocytes. Our samples consisted of fresh frozen sections that were unstained. Fitting the spectrum as an exponential decay between 500 and 1100 nm [mua = A*exp(-B*(lambda-C)) + D], we report on the fit parameters of and their variation due to biological heterogeneity as A = 4.20e4 +/- 1.57e5 [1/cm], B = 4.57e-3 +/- 1.62e-3 [1/nm], C = 210 +/- 510 [nm] , D = 613 +/- 534 [1/cm]. The variability in these results is likely due to highly heterogeneous distributions of eumelanin and pheomelanin.

8941-34, Session 9

Optical properties of human tissues in 400-2500 nm spectral range

Matija Milani?, Norwegian Univ. of Science and Technology (Norway); Ivar Skjåk Nordrum, St. Olavs Hospital (Norway); Lise Lyngsnes Randeberg, Norwegian Univ. of Science and Technology (Norway)

Optical methods are currently extensively exploited for diagnostic and therapeutic use in clinical medicine. Numerical simulations of light transport in tissue are important to understand, improve and develop these methods. Such simulations depend on reliable knowledge of the optical properties of human tissues.

Despite this fact, reliable sets of consistent optical data are lacking in literature. The available data is determined using a variety measurement procedures and wavelength ranges, which makes comparison of data difficult. The majority of existing data are measured on skin samples. Data on other human tissues are scarce for some spectral ranges significant for biomedical applications, e.g. the short wave IR region.

The aim of this study was to determine a complete set of optical parameters of human finger tissues. This is done to aid studies of human joint diseases. Samples of human skin, subcutaneous fat, skeletal muscle and tendons were collected during clinical autopsy. The samples were collected from 10 individuals. The study was approved by the regional ethical committee.

Double-integrating spheres were used to measure diffuse and collimated transmittance, and diffuse reflectance. In addition, diffuse transmittance and reflectance was measured in the wavelength range 400-2500 nm using hyperspectral imaging (HSI). HSI provided a mean to determine the spatial variation of the optical parameters. The inverse Monte Carlo algorithm (IMC) was used to determine absorption and scattering coefficients, and the anisotropy factor for each wavelength.

Results of our study provide consistent and reliable optical properties of human tissues important for the field of biomedical optics.

8941-35, Session 9

Determination of light scattering properties of thin slices of epithelial tissue based on three-dimensional refractive index mappings of the tissue slices

Jing-Wei Su, Wei-Chen Hsu, Kung-Bin Sung, National Taiwan Univ. (Taiwan)

Light scattering properties of epithelial tissues, including scattering phase functions, scattering cross-sections and scattering coefficients, are important parameters for optical diagnostic techniques. These properties are associated with the subcellular structures of tissues and could serve as indicators of pathological states of tissues. Experimentally, these properties are difficult to be determined accurately. We synthesized numerical epithelial tissue models by using experimentally-measured three-dimensional (3D) refractive index distributions of suspended and adherent epithelial cells. The nucleus-to-cytoplasm ratios of normal and dysplastic tissues were set according to the literature. Our simulation results from artificial tissue slices demonstrate that dysplastic tissues present significantly higher total scattering cross-sections and scattering coefficients than normal tissues. Furthermore, we quantified 3D refractive

index distributions of thin slices of normal and cancerous human oral mucosa specimens. Scattering cross-sections and scattering coefficients were estimated by the first order Born's approximation. The results characterize subtle subcellular structures of the normal and cancerous epithelial tissues and elucidate the correlation between the structures and their light scattering properties. These light scattering properties can serve as quantitative biomarkers for early cancer diagnosis and are important parameters for modeling photon propagation in soft tissue.

8941-36, Session 9

Optical signature of multiCellular tumor spheroid using index- mismatch- induced spherical aberrations

Corinne Lorenzo, Gwenaéle Le Corre, Pierre Weiss, Bernard Ducommun, Institut des Technologies Avancées en Sciences du Vivant, CNRS (France)

The development of new cancer treatments and the early prediction of their therapeutic potential are often made difficult by the lack of predictive pharmacological models. The 3D multicellular tumor spheroid (MCTS) model offers a level of complexity that recapitulates the three-dimensional organization of a tumor and appears to be fairly predictive of therapeutic efficiency. The use of spheroids in large-scale automated screening was recently reported to link the power of a high throughput analysis to the predictability of a 3D cell model. The spheroid has a radial symmetry; this simple geometry allows establishing a direct correlation between structure and function. The outmost layers of MCTS are composed of proliferating cells and form structurally uniform domain with an approximate thickness of 100 microns. The innermost layers are composed of quiescent cells. Finally, cells in the center of the spheroid can form a necrotic core. This latest region is structurally heterogeneous and is poorly characterized. These features make the spheroid a model of choice and a paradigm to study the optical properties of various epithelial tissues.

In this study, we used an in-vitro optical technique for label-free characterization of multicellular systems based on the index- mismatch induced spherical aberrations. We achieve to monitor and characterize the optical properties of MCTS. This new and original approach might be of major interest for the development of innovative screening strategies dedicated to the identification of anticancer drugs.

Conference 8941B: Terahertz and Ultrashort Electromagnetic Pulses for Biomedical Applications

Sunday 2 –2 February 2014

Part of Proceedings of SPIE Vol. 8941 Optical Interactions with Tissue and Cells XXV; and Terahertz for Biomedical Applications

8941-49, Session Key

THz impulse radar for biomedical sensing *(Keynote Presentation)*

Elliott R. Brown, Wright State Univ. (United States); Shijun Sung, Warren S. Grundfest M.D., Zachary D. Taylor, Univ. of California, Los Angeles (United States)

The THz impulse radar is an “RF-inspired” sensor system that has performed remarkably well since its development nearly six years ago. It was developed for ex vivo skin-burn imaging, and has since shown great promise in the sensitive detection of hydration levels in soft tissues of several types, such as ex vivo corneal samples. An intriguing aspect of the impulse radar is its hybrid architecture which combines the high-peak-power of photoconductive switches with the high-responsivity and -bandwidth (RF and video) of Schottky-diode rectifiers. The result is a very sensitive sensor system which is quasi-coherent in the sense that its signal-to-noise ratio varies approximately linear with the integration time, but the phase information is discarded, which is beneficial in mitigating the effects of clutter and speckle. This talk will summarize studies done on the optimization of THz impulse radar using optical (Gaussian-beam) and large-signal-processing (MATLAB) analysis. A system-performance example will be presented for corneal hydration sensing, and the inherent affordability of this system compared to existing THz spectrometric (time- and frequency-domain) instruments will be emphasized.

8941-65, Session Key

Investigation of the Frohlich hypothesis with high intensity terahertz radiation *(Keynote Presentation)*

Peter Weightman, Univ. of Liverpool (United Kingdom)

Many decades ago Herbert Frohlich hypothesised [1] that long wavelength (THz) modes play an important role in biological self organization and mediate the formation of a coherent state. Frohlich argued that the self-organization of living systems is maintained by a flow of free energy through a coherent exited state maintained by metabolic processes. He predicted that under appropriate conditions biological systems can support coherent excitations in the range 109 to 1012 Hz. This hypothesis is very relevant to the question as to whether or not quantum mechanics plays a non-trivial role in living systems an idea that has been discussed by physicists for decades and largely dismissed by biologists because there is no conclusive experimental evidence [2].

This talk will present the results of a recent investigation of the Frohlich hypothesis using high intensity THz radiation from the ALICE energy recovery linear accelerator at the Daresbury laboratory [3]. ALICE is a 35MeV energy recovery linear accelerator, the third of its kind in the world and the only one in Europe. It provides an intense source of pulsed THz radiation that is delivered to a tissue culture facility cleared for work on cancer. An account will be given of a recently funded research programme which will develop THz instruments on ALICE for the study of cancer in association with the scanning near field microscope (SNOM) on the infrared free electron laser on ALICE. The latter yields chemical specific images with a spatial resolution of 0.1 micron [4].

1 H. Frohlich H Int. J. Quantum Chem. 2 641 (1968)

2 P. Weightman, Physical Biology. 9 053001 (2012) (Review)

3 R.L. Williams et. al. Phys. Med. Biol. 58 373 (2013)

4 A. Smith et al App. Phys. Lett. 102 53701(2013)

8941-50, Session 10

Effect of intense THz pulses on expression of genes associated with skin cancer and inflammatory skin conditions *(Invited Paper)*

Lyubov V. Titova, Ayesheshim K. Ayesheshim, Univ. of Alberta (Canada); David Purschke, Univ of Alberta (Canada); Andrey Golubov, Rocio Rodriguez-Juarez, Rafal Woycicki, Univ. of Lethbridge (Canada); Frank A. Hegmann, Univ. of Alberta (Canada); Olga Kovalchuk, Univ. of Lethbridge (Canada)

Progress in the development of broadband terahertz (THz) pulse sources has inspired many novel applications of THz pulses in biomedical imaging. At the same time, we are only starting to uncover the effects of pulsed THz radiation, and especially intense THz pulses on biological tissue. Recently, we demonstrated that intense, picosecond THz pulses induce phosphorylation of H2AX, indicative of DNA damage, and at the same time activate DNA damage response in artificial human skin tissues [1]. We have also shown that intense THz pulses have profound impact on global gene expression in human skin. The distribution of intense-THz-pulse-sensitive genes is not uniform across the genome, with a significant number of affected genes belonging to the epidermal differentiation complex (EDC) in the 1q21 locus [2]. EDC genes encode for proteins that participate in epidermal differentiation and are often overexpressed in skin cancer and inflammatory skin conditions such as psoriasis and atopic dermatitis. The observed THz-induced changes in expression of EDC genes and other genes implicated in non-melanoma skin cancers and inflammatory skin disorders are in many cases opposite to disease-related changes, suggesting the potential application of intense THz pulses for treatment aimed at normalizing the expression of these disease-related genes.

[1] L. V. Titova, A.K. Ayesheshim, A. Golubov, D. Fogen, R. Rodriguez-Juarez, F. A. Hegmann, and O. Kovalchuk, Biomed Opt Express 4, 559 (2013).

[2] L. V. Titova, A.K. Ayesheshim, A. Golubov, R. Rodriguez-Juarez, R. Woycicki, F. A. Hegmann, and O. Kovalchuk, Sci Rep 3, 2363 (2013).

8941-52, Session 10

Terahertz spectroscopy for classification of burn wounds in a standardized porcine model *(Invited Paper)*

M. Hassan Arbab, Samuel C. Henry, Adelaide Warsen, Dale P. Winebrenner, Abbi M. McClintic, Anne M. Hocking, Nicholas Shubin, Saman Arbabi M.D., Univ. of Washington (United States)

Noninvasive delineation of burn wounds remains a significant challenge, especially when access to highly-trained burn specialists is not available. Burns wounds can be stratified, according to the depth of the compromised tissue, into four clinically useful grades: epidermal, superficial partial-thickness, deep partial-thickness and full-thickness. While superficial partial-thickness burns can naturally heal without surgical intervention, full-thickness burns require excision and skin grafting procedures. Deep partial-thickness burns, however, can worsen and reach full-thickness depth over time. We present experimental results from the application of terahertz time-domain spectroscopy in differentiation of superficial partial-thickness, deep partial-thickness and full-thickness burns in a standardized porcine model. Porcine burn

models are clinically more accurate, and therefore are favored over other animal models such as rodents, because the skin regeneration in swine more closely represents the wound healing process in humans. We followed a previously standardized in vivo protocol that consistently creates scald burns of clinically relevant severity levels. We used various histological assays to quantify the severity of burns according to the depth of the damaged skin by observing different biological viability markers. Statistical analysis of our results (n=9) indicate that the terahertz spectral information of the wound between 0.2 and 0.8 THz can be used to discriminate between burns of varying severity that will require different clinical treatment plans.

8941-63, Session 10

The potential of THz radiation to perturb and manipulate biological function

George Peter Swift, Andrew J. Gallant, Durham Univ. (United Kingdom); G. J. Sharples, Durham Univ. (United States); John Martyn Chamberlain, Durham Univ. (United Kingdom)

There have been persistent reports that THz irradiation of plants, cells, tissues and even animal organisms may affect growth rates, cell damage and healing processes. Mounting evidence suggests that radiation-induced gene instabilities in structures with the lowest level of biological organization, and under controlled temperature conditions, may be responsible for such phenomena. In turn, the physical cause of such genetic instabilities (e.g. over- or under-expression of specific genes) may involve the presence of long-lived excitations in the vibrational spectrum of DNA, leading to local unzipping of DNA ('bubbles') and modifications of transcription processes. In practical terms, the THz manipulation of such processes might deliver therapies, controlled stem cell differentiation and novel DNA synthesis.

In this review, we shall summarize the experimental evidence that athermal processes occur in biological systems. We shall then review the fundamental physics of the 'bubble' phenomenon, as parts of the DNA chain adopt configurations far from equilibrium. We shall then describe our current investigations into the flexibility of small sections of DNA under THz irradiation, and how these may be related to the presence of bubbles. Finally we shall consider a number of possible future directions for the field, especially in investigations of bacteria.

8941-64, Session 10

State-of-the-art exposure chamber for highly controlled and reproducible THz biological effects studies

Cesario Z. Cerna, General Dynamics Advanced Information Systems (United States); David P. Elam, Air Force Research Lab. (United States); Ibtissam Echchgadda, Mark A. Sloan, General Dynamics Advanced Information Systems (United States); Gerald J. Wilmink, Air Force Research Lab. (United States)

Terahertz (THz) imaging and sensing technologies are increasingly being used in a host of medical, military, and security applications. For example, THz systems are now being employed at international airports for security screening purposes and at major medical centers for cancer and burn diagnosis. Recent advances in THz applications have stimulated interest regarding the biological effects associated with this frequency range. THz biological effects research is therefore required for the safe use of THz systems, identification of health hazards, and development of empirically-based safety standards. In this study, we developed a state-of-the-art exposure chamber that allows for highly controlled and reproducible THz bioeffects studies. This innovative system incorporates an industry grade cell incubator system that permits for a highly controlled exposure environment, where temperatures can be maintained at 37°C ± 0.1 °C, CO2 levels at 5% ± 0.1%, and relative humidity levels at 95% ± 1%. To maximize the THz power transmitted to the cell culture region inside the humid incubator, a secondary custom micro-chamber was fabricated and incorporated in the system. This micro-chamber is shielded from the incubator environment and can be nitrogen-purged to eliminate water absorption effects. Additionally, a microscope that allow for live-time visualization of the cells before, during, and after THz exposure was integrated into the exposure system.

8941-53, Session 11

Multimodal terahertz pulsed and polarization optical imaging for delineating nonmelanoma skin cancers

Anna Yaroslavsky, Univ. of Massachusetts Lowell (United States) and Massachusetts General Hospital, Boston, MA (United States); Melissa Spencer, Univ. of Massachusetts Lowell (United States); Maxim Evdokimov, Teraimaging Inc. (United States); Alexander P. Shkurinov, Lomonosov Moscow State Univ. (Russian Federation); Victor Neel, Massachusetts General Hospital (United States)

We investigated combining Terahertz Pulsed Imaging (TPI) with polarization enhanced reflectance optical imaging for the delineating nonmelanoma skin cancers (NMSC). Fresh thick samples of skin tissue, with and without tumor, were obtained several hours after surgery. The samples were mounted in a sample holder and imaged in optical and THz regimes, without remounting between the imaging procedures. The images were then overlaid so that the optical images could verify observations made by TPI. Analysis was performed on a pixel-by-pixel basis in both the time and frequency domains. Both TPI and optical images were evaluated against histopathology. Several analysis techniques were applied to compare the shape of the terahertz time domain pulses between diseased and normal tissue. In the frequency domain, the power spectra were compared to evaluate additional methods of emphasizing contrast. Our results show differences in reflection of terahertz radiation from diseased and normal tissues. The pulses reflected from the diseased tissues are elongated in time. While TPI has demonstrated contrast between diseased and normal tissue, it can also highlight tissues that are normal, but with different density and / or water content. TPI alone lacks the resolution necessary to distinguish between these types of tissues. Combining TPI with optical imaging allows for classification of these morphological structures. TPI shows the ability to highlight potential tumor sites, which can then be examined with higher resolution in the optical regime. Combined THz and optical imaging has the potential for quick intraoperative delineation of cancers.

8941-54, Session 11

Portable terahertz spectroscopy system for the noninvasive assessment of skin burn severity

Mark A. Sloan, Cesario Z. Cerna, General Dynamics Advanced Information Systems (United States); David P. Elam, Air Force Research Lab. (United States); Ibtissam Echchgadda, General Dynamics Advanced Information Systems (United States); David M. Burmeister, Robert J. Christy, U.S. Army Institute of Surgical Research (United States); Gerald J. Wilmink, Air Force Research Lab. (United States)

Skin burns are characterized by depth of injury into four clinical categories: superficial (1st degree), superficial-partial thickness (2nd degree), deep-partial thickness (2nd degree), or full thickness burns (3rd

degree). Clinicians can use visual assessment methods to accurately diagnose 1st and 3rd degree burns. However, such approaches cannot be used to accurately differentiate between visually similar superficial-partial and deep-partial thickness 2nd degree burns, thereby requiring more invasive techniques. Since the wound conversion rates differ dramatically between 2nd degree burn types, each type of wound requires a different treatment in order to achieve an optimal outcome. As a result, definite clinical tools are needed to non-invasively ameliorate diagnosis and monitoring of skin burn injuries. Skin burns typically exhibit higher water concentrations (edema) following a burn. These edema levels rise with wound severity. In this study, we tested the performance of a compact terahertz time-domain spectroscopy (THz-TDS) system for detecting small differences in water edema levels in skin burn injuries. A graded burn severity protocol was applied in an in vivo porcine model, and injuries were evaluated for four days post burn using the THz-TDS device, multiprobe skin system, and infrared thermographic imaging. Optical properties for each skin burn were collected from 0.1 to 1.6 THz. The multiprobe adapter system was used to quantify each burn's hydration level, transepidermal waterloss hemoglobin and melanin. Burn severity was verified by histological analyses performed on tissue biopsies. The results show that portable THz spectrometers are useful, practical tools for the noninvasive assessment of skin burn injuries.

8941-55, Session 11

In vivo analysis of cellular-level inflammatory response induced by pulsed THz irradiation

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Recent development of various THz source providing wide range of frequency and power leads to increasing interests in THz-wave enabled applications. Especially, biomedical applications have been continuously pursued owing to unique characteristics of THz wave such as strong interaction with water, non-ionizing energy level and molecular sensitivity. Thermal effects and tissue damage induced by intensive CW THz irradiation have been well-characterized by recent in vitro and ex vivo studies. However, in vivo analysis of cellular-level effects by moderate THz irradiation with negligent thermal effects have been rarely performed. In this study, we monitored cellular-level inflammatory response caused by pulsed THz irradiation in live mouse model in vivo. With FEL based pulsed THz source, we irradiated anesthetized mouse ear skin by pulsed THz wave (2.7THz, 3?s pulsewidth) with average power of 80mW/cm2 for 30 minutes. In contrast to in vitro analysis using cultured cell at this power level of CW THz radiation, we couldn't observe any noticeable thermal effects in the irradiated mouse ear skin. For in vivo analysis of cellular-level effects in microscopic scale, we utilized custom-built laser-scanning confocal microscopy system. For repetitive imaging before and after THz irradiation, we identified specific blood vessel pattern of ear skin to locate same skin area by using genetically engineered mouse exclusively expressing green fluorescence at blood vessel. To monitor inflammatory response, we imaged behaviors of neutrophils as first-responders migrating towards the site of inflammation. We observed massive recruitment of neutrophils to the THz irradiated skin area within 8 hours.

8941-56, Session 12

TBA *(Invited Paper)*

P. Thomas Vernier, The Univ. of Southern California (United States); Richard Nuccitelli, BioElectroMed Corp. (United States)

No Abstract Available

8941-57, Session 12

Nanosecond pulsed electrical fields generate measurable pressure transients

Caleb C. Roth, The Univ. of Texas Health Science Ctr. at San Antonio (United States); Saher Maswadi, The Univ. of Texas San Antonio (United States); Bennett L. Ibey, Hope T. Beier, Gary L. Thompson III, Air Force Research Lab. (United States); Erick K. Moen, The Univ. of Southern California (United States); Randolph D. Glickman, The Univ. of Texas Health Science Ctr. at San Antonio (United States)

Nanosecond pulsed electrical fields (nsPEF) cause multiple cellular effects ranging from acute changes in structure and morphology to transient increases in intercellular calcium and, finally, to changes in gene expression associated with intercellular signaling pathways. Interestingly, researchers studying the propagation of pressure waves in an aqueous environment have used nsPEF in the past to create pressure transients (shockwaves) for study. Mechanical transduction in mammalian cells is accomplished via transient receptor potential (TRP) channels, integrins, and stretch-activated channels (SACHs channels). Other membrane-bound channels, such as voltage-gated and ligand-gated channels, have also been shown to be secondarily activated by mechanical forces. As little as 50pN of force applied to a cell has been shown to activate the Notch signaling pathway. We hypothesize that nsPEF effects could be directly related to mechanical stress from pressure transients, which are sufficient to trigger the activation of intercellular signaling pathways and to cause the mechanical activation of voltage and ligand-gated channels, thus allowing the free passage of ions. To quantify the amount of mechanical force generated at the cell, we used probe beam deflection to measure pressure transients generated by a typical nsPEF exposure. We observed that nsPEF generate shockwaves that interact with the plasma membrane. It remains unclear whether this pressure transient can cause the observed changes in the cells following nsPEF, but it does highlight the necessity to either control for, or account for, the additive effects of both electrical and mechanical stressors present during nsPEF stimulation.

8941-58, Session 12

Nonlinear imaging techniques for the observation of cell membrane nanoporation due to exposure to nanosecond pulsed electric fields

Erick K. Moen, Univ. of Southern California (United States); Hope T. Beier, Air Force Research Lab. (United States); Caleb C. Roth, The Univ. of Texas Health Science Ctr. at San Antonio (United States); Gary Thompson, Oak Ridge Institute for Science & Education (United States); Bennett L. Ibey, Air Force Research Lab. (United States)

Traditional electroporation of biological membranes utilizing electric pulses lasting hundreds of microseconds has become a popular biomedical tool for transporting material such as DNA or drugs into a cell. Recently, research into the application of nanosecond pulsed electric fields (nsPEF) to various cell lines has demonstrated that these much shorter pulses are also capable of causing repairable damage to plasma membranes. This agitation manifests itself as increased membrane permeablization. Molecular dynamic simulations have been used to provide insight as to the genesis of this phenomenon by studying the reaction of lipid bilayers to intense electric fields. These simulations depict the formation of aqueous pores, leading to the assumption that this permeablization is due to the creation of nanopores in the membrane. Verifying the nature and existence of these pores experimentally, however, presents a number of challenges and has proved elusive thus

far. This report focuses on two unique methodologies for accomplishing this task and provides experimental data to corroborate current theories on electroporation in the nanosecond regime. The first imaging technique discussed herein uses probes capable of second harmonic generation to exploit the normally ordered nature of the membrane and contrast it to its permeablized state. The second utilizes the sub-diffraction limit imaging capabilities of stimulated emission depleted (STED) microscopy to look more closely at the event as it occurs. Experiments were performed on both biological cells and giant unilaminar vesicles (GUVs) to explore the physics of the process with and without further biological confounders.

8941-59, Session 12

AC field induced cell membrane temperature gradients *(Invited Paper)*

Allen L. Garner, Purdue Univ. (United States); Maxim Deminsky, Russian Research Ctr. Kurchatov Institute (Russian Federation); Bogdan Neculaes, GE Global Research (United States); Boris Potapkin, Russian Research Ctr. Kurchatov Institute (Russian Federation)

Despite the absence of bulk heating in many biomedical applications of electric fields, such as electroporation, much speculation exists concerning the impact of temperature gradients that may arise due to short electric pulses, particularly those of submicrosecond duration. We recently derived analytic expressions for temperature changes across biological cells exposed to pulsed electric fields and benchmarked the results to one- and three-dimensional finite element simulations on nanosecond and microsecond time scales (A. L. Garner, et al., J. Appl. Phys. 113, 214701 (2013)). The analyses showed that shorter pulses induced higher temperature gradients on shorter timescales than longer pulses. Alternatively, one may apply AC fields, with frequencies ranging from kilohertz to terahertz, to modify or measure biological cells and tissues. Temperature gradients induced by AC fields are especially interesting given reports of microwave induced membrane permeabilization with membrane voltage below the threshold for traditional electroporation. The main practical difference between AC fields and unipolar pulses (e.g. square pulses) is the repetition rate, which is typically much lower for unipolar pulses (on the order of 10 Hz) than the equivalent parameter for AC fields. Because the relationship between repetition rate (or frequency for AC fields), pulse duration, and the thermal diffusion time plays a critical role in the development of the cell membrane temperature gradient, we calculate the temperature gradient generated at various frequencies, compare the results qualitatively to unipolar pulsed electric field induced gradients, and hypothesize about the significance for biomedical applications.

8941-60, Session 12

Effects of nanosecond pulsed electrical fields (NSPEFS) on the cell cycle of CHO and Jurkat cells

Megan A. Mahlke, U.S. Air Force (United States); Christopher Navara, The Univ. of Texas at San Antonio (United States); Bennett L. Ibey, U.S. Air Force (United States)

Exposure to nano-second pulsed electrical fields (nsPEFs) can cause poration of external and internal cell membranes, DNA damage, and disassociation of cytoskeletal components, all of which are capable of disrupting a cell's ability replicate. Variations between cell lines in membrane and cytoskeletal structure as well as in survival of nsPEF exposure should correspond to unique line-dependent cell cycle effects. Additionally, phase of cell cycle during exposure may be linked to differential sensitivities to nsPEFs across cell lines, as DNA structure, membrane elasticity, and cytoskeletal structure change dramatically during the cell cycle. Populations of Jurkat and Chinese Hamster Ovary (CHO) cells were examined post-exposure (10 ns pulse trains at 100, 150, or 200kV/cm) by analysis of DNA content via propidium iodide staining and flow cytometric analysis at various time points (1, 6, and 12h post-exposure) to determine population distribution in cell cycle phases. Growth curves of both cell lines post-nsPEF exposure were also analyzed. CHO populations exhibited reversible arrest in G2/M phase that increased in severity with increasing dose; however, Jurkat cells did not appear to exhibit phase-specific arrest. Arrest of CHO cells in G2/M phase suggests that nsPEFs inhibit the cell's ability to complete mitosis, perhaps via inhibiting microtubule formation. Interestingly, CHO cells have a more robust and rigid cytoskeleton than Jurkat cells which is thought to contribute to their ability to survive nsPEFs. The ability of the CHO cytoskeleton to recover and complete mitosis after nsPEF-induced damage may be integral to the cell line's higher tolerance of nsPEF exposure.

8941-61, Session 12

Investigation of a direct effect of nanosecond pulse electric fields on mitochondria

Larry E. Estlack, General Dynamics (United States); Cesario Z. Cerna, General Dynamics Advanced Information Systems (United States); Caleb C. Roth, General Dynamics Information Technology (United States); Gerald J. Wilmink, Air Force Research Lab. (United States); Bennett L. Ibey, U.S. Air Force (United States)

The unique cellular response to nanosecond pulsed electric field (nsPEF) exposure, as compared to longer pulse exposure, has been theorized to be due to permeabilization of intracellular organelles including the mitochondria. In this paper, we utilize a high throughput oxygen and pH sensing system (Seahorse XF24 extracellular flux analyzer) to access the mitochondrial activity after nsPEF exposure in two cell lines, Jurkat and U937. The XF Analyzer creates a transient micro-chamber of only a few microliters in specialized cell culture micro plates. This enables oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) to be monitored in real time. We found that for 10 ns exposures at 50 kV/cm, 10 and 30 pulses caused an increase in U937 metabolic capacity in OCR and ECAR. However, at 100 pulses and for higher amplitude exposure (150 kV/cm) we found a significant decrease in OCR and ECAR as compared to unexposed sham populations. In Jurkat cells, we found that previous reports of sensitivity difference amongst cells were also present using this measurement technique. These results suggested that the exposures were modulating metabolic activity in cells, possibly due to direct effects on the mitochondria themselves. To validate this hypothesis, we isolated mitochondria from U937 cells and exposed them similarly and found no significant change in metabolic activity for any pulse number. These results suggest that direct permeabilization of the mitochondria is unlikely a dominant effect of nsPEF exposure and intracellular pathway activation leading to mitochondrial depolarization is likely responsible for observed pulse-induced mitochondrial effects.

8941-62, Session PSun

Dose-dependent translocation of fluorescent probes of PIP2 hydrolysis in cells exposed to nanosecond-pulsed electric fields

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Nanosecond pulsed electric fields (nsPEF) have been shown to stimulate intracellular pathways akin to pharmaceutical treatment, but without

specific membrane receptor dependence. This observation suggests that nsPEF may be useful across an array of biomedical applications including pain management and tissue repair. Previously, we found that one 600ns 16.2 kV/cm electric pulse caused initiation of complex plasma membrane derived intracellular lipid signaling cascade similar to Gq/11 receptor mediated phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4) P2) hydrolysis. By reducing the width of the pulse by 10x (60ns, 16.2 kV/cm), we observed a slower, and smaller amplitude response. By increasing number of 60ns pulses to 20 (delivered at 5 Hz) the speed and intensity of fluorescent changes were increased to the level of single 600 nsPEF exposure. These observations suggest that there is a “dose” dependency to nsPEF-induced activation of intracellular pathways and this dependency appears to parallel dependencies observed previously while studying nsPEF-induced membrane permeabilization using whole cell patch clamp technique. The mechanism of action triggered by nsPEF exposure culminating in the activation of intracellular pathways remains unclear, but understanding the fundamental “dose” dependency shows that simply increasing the exposure number will enhance the effect. This points to the lack of a direct dependence of the reaction on the peak electric field and suggests duration and number of exposures indeed matter. Future efforts will focus on modification of the cellular environment to determine what chemical species is being enhanced upon successive pulsing unpinning this dose dependency.

Conference 8942:
Dynamics and Fluctuations in Biomedical Photonics XI

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8942 Dynamics and Fluctuations in Biomedical Photonics XI

8942-1, Session 1

Tracking dynamic optical vortices using a minimum mean square error estimator *(Invited Paper)*

Dennis Thomas, Sean J. Kirkpatrick, Michigan Technological Univ. (United States)

Optical vortices are phase singularities found in the 2-dimensional phase of scattered fields. They are readily localized based on the fact that the phase rotates through a full 2??radians in a closed loop surrounding the singularities. Vortices appear only in pairs of oppositely 'charged' vortices in which the phase rotation is either in the clockwise or counter clockwise direction. In a dynamic speckle field, the spatial coordinates of the vortices change with time as the speckle pattern evolves. By tracking the location and lifetimes of the vortex population through time, the dynamics of the scattering medium may be inferred. We present a method based on minimizing the mean square error between sequential vortex fields for tacking and arriving at lifetime statistics for vortices arising from scattering media with varying temporal behaviors. The approach presented is a nearest neighbor algorithm that accounts for vortex pair annihilation and vortex pair creation. We will present both computational and experimental data demonstrating the effectiveness of our approach at determining the lifetime of vortices and showing how the lifetime of the vortices is shorter for a rapidly moving scattering medium vs. a more slowly moving medium. From the lifetime statistics of the vortices, we will show how probability of survival distributions can be formulated and how these PDF's can be used to infer the behavior of the dynamic scattering medium.

8942-2, Session 1

Deep tissue blood perfusion during cold-induced vasodilation using diffuse speckle contrast analysis

Renzhe Bi, Jing Dong, Kijoon Lee, Nanyang Technological Univ. (Singapore)

Recently, diffuse speckle contrast analysis (DSCA) is developed for deep tissue blood perfusion study. Comparing with existing methods, such as contrast-enhanced CT and MRI, DSCA is noninvasive and non-radioactive with much flexible instrument. Comparing with other optical methods, such as diffuse correlation spectroscopy (DCS), DSCA is more cost effective and has much easier analysis algorithm. We build a multi-channel DSCA device with fiber based probes. Our probe can stick to skin firmly and work properly under a wide range of conditions, such as low temperature.

We use this DSCA device for blood perfusion measurement during cold-induced vasodilation (CIVD). CIVD happens after cold exposure, possibly to protect tissue from cold injury. When part of human skin is exposed to cold, vasoconstriction will occur first, result in fast cooling of tissue. However after about 5 minutes vasodilation will occur to increase the blood flow, and the temperature of that part of skin will also increase subsequently. Then a new phase of vasoconstriction and vasodilation will repeat. CIVD is affected by diet, alcohol consumption, altitude, age and stress. This phenomenon can be used to study Raynaud's disease.

People usually study CIVD by measuring only the skin temperature. Some groups take both temperature and skin surface blood perfusion by laser Doppler Flowmetry on one hand. We measure both hands' temperature and blood perfusion simultaneously during CIVD in one hand, with the other one as reference, taking advantage of the flexible probes of the DSCA setup. The correlation of the increase of blood flow and skin temperature during CIVD is properly studied. Comparisons between two hands are demonstrated.

8942-5, Session 1

Spatial and temporal integration for dynamic speckle contrast calculation

Julio C. Ramirez-San-Juan, INAOE (Mexico); Bernard Choi, Beckman Laser Institute and Medical Clinic (United States); Gabriel Martinez-Niconoff, Ruben Ramos-Garcia, INAOE (Mexico)

Different speckle-based methods have been developed to characterize tissue blood flow and perfusion. One such method, called Laser Speckle Imaging (LSI) [1], enables computation of blood flow maps with relatively high spatial and temporal resolution. One of the major limitations of LSI is a reliable measurement of the correlation time (?C) and, hence, the blood's speed on blood vessels. This limitation has been attributed mainly to the fact that LSI is based on an approximate model [2]. It is well known that speckle contrast measurement is susceptible to contrast reducing effects like spatial averaging due to a finite size of the CCD pixel with which speckle images are acquired. In this work, we present a new model expression for integrated speckle contrast which accounts for temporal and spatial integration due to the finite size of the pixel of the CCD camera; as a result we found that a correction factor should be introduced to the measured speckle contrast to properly determine ?C. Experimental data are shown that support our theoretical model.

[1] A. Fercher and J. Briers, "Flow visualization by means of single-exposure speckle photography", Opt. Commun. 37, 326-330 (1981).

[2] D.D. Duncan and S.J. Kirkpatrick, "Can laser speckle flowmetry be made a quantitative tool?", JOSA A, 25, 2088-2094 (2008)

8942-6, Session 1

Polarization analysis of scattering for effective particle sizing in laser speckle rheology

Zeinab Hajjarian Kashany, Seemantini Nadkarni, Harvard Medical School (United States)

Progression of numerous pathologies is concurrent with alteration of tissue mechanical properties. This highlights the demand for optical diagnostic tools that enable label free, non-contact, and non-destructive evaluation of tissue mechanical properties, in its native state. Laser Speckle Rheology (LSR) has been developed to fulfill this requirement. In LSR tissue is illuminated by a laser beam and the temporally fluctuating speckle patterns are acquired with a high speed camera. Speckle intensity auto-correlation function, g2(t), is evaluated from speckle frames, the mean square displacement (MSD) of scattering particles is extracted, and the viscoelastic modulus, G*(?) is derived via the Generalized Stokes Einstein Relation (GSER). Since the MSD of scattering particles is intimately related with scattering particle size, this information is necessary for accurate analysis of G*(?) in LSR. In this study, we exploit the radial and polar remittance profiles of intensity in parallel and perpendicular polarization states for estimation of effective scattering particle size. Our experimental results obtained from coagulating blood specimens reveal that changes in scattering particle

size distribution induce striking changes in the lobular intensity patterns obtained through polarization filtering of back-scattered intensity. In the cross-polarized channel, the full width half maximum (FWHM) of lobes is inversely proportional to the effective particle size. At the same time, the co-polarized component evolves from an elliptical form to a four-lobe pattern with increasing particle size. Our preliminarily Polarized Monte-Carlo simulations support the experimental observations and show that the FWHM of intensity lobes in cross-polarized channel for scattering particle of 0.1 and 1 ?m radii are equal to 80o and 50o, respectively.

8942-8, Session 2

Liquid crystal-based spectral imaging goniometric polarimeter for sample characterization

James C. Gladish, Donald D. Duncan, Portland State Univ. (United States)

The ability to measure polarization effects is important in many biological and industrial applications. Additionally, measuring spectral and scatter effects can offer greater sensitivity for applications where characterization and differentiation are important. Here we present a liquid crystal-based spectral imaging goniometric polarimeter to probe these effects. The system consists of two modules, a Stokes generator and a polarimeter, each constructed from a pair of liquid crystal variable retarders (LCVR). LCVRs are computer-controlled birefringent devices that impart retardance effects with no mechanical movement. Additionally, the Stokes generator utilizes a computer-controlled liquid crystal tunable filter (LCTF) to transmit a specific wavelength bandwidth, also with no mechanical movement. The polarimeter, enclosed in a cage system, manually rotates around the sample plane to provide angular scatter measurements. A CCD camera images the sample and provides spatially resolved estimates of the complete Mueller matrix as a function of wavelength and scatter angle. Here we describe the system and its calibration, and show quantitative measurement results for a number of samples.

8942-9, Session 2

Voice coil based robust and miniature optical delay for multiple reference optical coherence tomography

Roshan I. Dsouza, Kai Neuhaus, National Univ. of Ireland, Galway (Ireland); Josh Hogan, Carol Wilson, Compact Imaging, Inc. (United States); Martin J. Leahy, Hrebesh Subhash, National Univ. of Ireland, Galway (Ireland)

Multiple reference optical coherence tomography (MRO™) is a recently developed miniature time-domain low coherence interferometeric imaging platform, which promises to fit into robust, cost-effective design: virtually solid state, typical of handheld devices. The key element of MRO is a multiple references optical delay based on piezoceramic (PZT) and a partial mirror. However, the non-linear operation and limited axial displacement range at higher scanning frequency are the major limitations of PZT based optical delay. Moreover, PZT based actuators require relatively high operational voltage. In this paper we present a novel optical delay based on voice coil actuator for MRO™. Voice coil based actuators offer numerous advantages such as liner operation, zero hysteresis, low operational voltage, long life, light weight and inexpensive. We demonstrate multiple reference optical delay based on voice coil can be a good alternative to PZT based optical delay, which can provide fast and still sufficient, precise axial displacements at high scanning rate.

8942-10, Session 2

Improvement of tissue analysis and classification using optical coherence tomography combined with Raman spectroscopy

Chih Hao Liu, Ji Qi, Shang Wang, Chen Wu, Wei Shih, Kirill Larin, Univ. of Houston (United States)

Fast and accurate tissue characterization is required for the prediction and diagnosis of multiple diseases. Optical coherence tomography (OCT) has been demonstrated to be an effective high-resolution imaging tool for characterizing the tissue types based on their optical properties, e.g. refractive index and scattering coefficient. However, the resemblance of the microscopic and optical characteristics of tissues requires the combined imaging modalities for advanced assessment of tissue types. Raman spectroscopy (RS) provides the information about the chemical composition of tissue at the molecular level. Here, we report a two-dimensional computational method which combines the slope of OCT intensity signal with the Principal component (PC) of RS. Based on these two parameters, the classification of tissue types relies on the optical attenuation coefficient and the chemical ingredients of tissue. For the feasibility study, out pilot experiments were performed on mouse kidney, liver and small intestines (in vivo for OCT measurement and ex vivo for RS assessment). Results demonstrate a good differentiation among these three types of tissues with this combination. Also, an improvement of the tissue classification is observed compared with the analysis only based on the OCT detection (slope and standard deviation analyses of OCT intensity signal). With further development and more experiments on normal and pathological tissues, this combined OCT/RS method may potentially offer advanced optical biopsy of cancer and normal surrounding tissues.

8942-11, Session 2

Label free cell tracking in 3-D tissue engineering constructs with high resolution imaging

William A. Smith, Ka Po Lam, Katherine P. Dempsey, Keele Univ. (United Kingdom); James B. Richardson, Keele Univ. (United Kingdom) and The Robert Jones and Agnes Hunt Orthopaedic Hospital NHS Foundation Trust (United Kingdom); David M. Johnes, Ying Yang, Keele Univ. (United Kingdom)

Within the field of tissue engineering there is an emphasis on studying 3D biological structures. Consequently, to investigate and identify cellular activities and phenotypes in a 3D environment for all in vitro experiments, including shape, migration/proliferation and axon projection, it is necessary to adopt an optical imaging system that enables monitoring 3-D cellular activities and morphology through the thickness of the construct for an extended culture period without cell labelling. This paper describes a new 3-D tracking algorithm developed for Cell-IQ2®, an automated cell imaging platform, which has been equipped with an environmental chamber optimised to enable capturing time-lapse sequences of live cell images over a long-term period. As an integral part of the algorithm, a novel auto-focusing procedure was developed for phase contrast microscopy equipped with 20x and 40x objectives, to provide a more accurate estimation of cell growth/trajectories by allowing 3D voxels to be computed at high spatiotemporal resolution and cell density. A pilot study was carried out in a phantom system consisting of horizontally aligned nanofiber layers (with precise spacing between them), to mimic features well exemplified in cellular activities of neuronal growth in a 3D environment. This was followed by detailed investigations concerning axonal projections and dendritic circuitry formation in a 3D tissue engineering construct. Preliminary work on primary animal neuronal cells in response to chemoattractant and topographic cue within the scaffolds has produced encouraging results.

8942-12, Session 2

Laser scanning microscopic investigations of the penetration of nanocontainers for drug delivery through the skin barrier by tissue-tolerable plasma *(Invited Paper)*

Jürgen M. Lademann, Alexa Patzelt, Heike Richter, Charité Univ. Berlin (Germany); Olaf Lademann, Univ. Medicine Greifswald (Germany); Eckart Rühl, Freie Univ. Berlin (Germany); Grit Baier, L. Breucker, Katharina Landfester, Max-Planck-Institut für Polymerforschung (Germany)

For many years, several attempts have been made to enhance skin permeability by chemical, physical or mechanical manipulations to reduce the barrier function of the skin. The present study demonstrates the possibility of penetration enhancement of 400 nm sized nanocontainers loaded with a model drug consisting of a fluorescent dye by the application of tissue-tolerable plasma (TTP). Therefore, the stability of the nanocontainers and their penetration through the skin barrier prior to and in combination with TTP application was evaluated using laser scanning microscopy. The results revealed that the penetration of the nanocontainers could be effectively enhanced if applied in combination with TTP, hence delivering the model drug unaffected by plasma into deeper skin layers. The stability testing showed no significant structural changes of the nanocontainers after contact with TTP. Thus, the present study demonstrates the potential of laser scanning microscopic techniques for the development of new penetration strategies.

8942-13, Session 3

Tissue optics *(Invited Paper)*

Steven L. Jacques, Oregon Health & Science Univ. (United States)

No Abstract Available

8942-14, Session 4

Seawater virus detection and identification through light scattering and Brownian motion using full field interferometry *(Invited Paper)*

A. Claude Boccara, Benoit Queney, Institut Langevin (France); Martine Boccara, IBENS (France)

Viruses in marine environment have an ecological, geochemical and genetic impact, they affect algal blooms, nutrients cycling, microbial communities structure, the modulation of atmospheric gases and finally the transfer of genes between organisms. To get an insight into these observations, it is necessary to develop reliable methods to estimate the number and diversity of viruses in a given marine environment.

We have used the light scattered by individual active viruses to detect their number in a volume limited by the sectioning ability of our microscope (about 1 micrometer) and its field of view (about 100 micrometers) in order to count them non-destructively. More precisely we perform a shot noise limited measure of the interference between a reference beam and the light scattered by the viruses on a CCD camera. Up to now the setup is sensitive enough to measure the scattering level corresponding to virus radii of about 25 nanometers. The size and shape characterization is more precisely achieved by quantifying the translational Brownian motion.

The experimental results that have been carried out on a number of isolated sets of viruses (Chlorella virus, T4, T7, Lambda etc.) match the sizes found in the literature or that we can measure by electron microscopy. Mixture of viruses simulating real seawater sampling have also been studied and coupled to quantitative PCR amplification.

We are working to increase the sensibility in order to cover the full range of seawater viruses down to 15 nm radius.

8942-15, Session 4

Monte Carlo modeling of OCT-based microvascular imaging in skin

Alzbeta E. Hartinger, Stephanie A. Nam, Benjamin J. Vakoc, Wellman Ctr. for Photomedicine (United States)

Recent advances in optical coherence tomography (OCT) have proven its significant potential in dermatological research. Images of cutaneous microvasculature obtained by combining OCT with Doppler principles would considerably advance our understanding of capillary malformations, non-melanoma skin cancer growth, and tumor response to new treatments based on angiogenesis inhibitors. To assess and optimize OCT-based algorithms for imaging cutaneous microvasculature, a 3-D Monte Carlo model of the skin and its vascular network was developed. This modeling tool was developed in a C-language environment and offers the option to run on multithreaded CPUs or GPUs. The tool was also interfaced with Matlab for all data post-processing and image generation. The model simulates all desired skin layers and takes as an input a 3-D vascular network geometry that is incorporated into the dermis at the appropriate depth. The vascular network modeled with superellipsoids is automatically generated using predefined hypotheses characterizing blood vessel position, orientation, diameter, and length. The complete model of the sample allows simulation of 3-D conventional and vascular OCT datasets. Blood flow is modeled by randomly modulating the phase of photon packets with trajectories that include a scattering event within the boundaries of a vessel. Comparisons between acquired and modeled vascular images will be presented. The use of this modeling tool to both understand vascular OCT signals and to improve vascular algorithms will also be discussed. Furthermore, the extension of this model to non-skin sites will be described.

8942-16, Session 4

Photophysical properties and photodynamic efficiency of cationic porphyrins

Grigor V. Gyulkhandanyan, Institute of Biochemistry (Armenia); Robert K. Ghazaryan, Yerevan State Medical Univ. (Armenia); Marina H. Paronyan, Science and Production Ctr. Armbiotechnology (Armenia); Anna G. Gyulkhandanyan, Institute of Biochemistry (Armenia); Boris M. Dzhagarov, Institute of Molecular and Atomic Physics (Belarus); Elena S. Tuchina, Valery V. Tuchin, Saratov State Univ. (Russian Federation)

Photodynamic inactivation of some microorganisms (St. aureus, E.coli) was investigated and their dependence on photo-physical properties of photosensitizers (PS) (cationic porphyrins and metalloporphyrins) was shown. One of the most important criteria for the effectiveness of the PS`s is the quantum yield of singlet oxygen (?Δ). Our investigations were shown that ?Δ of metalloporphyrins, containing Zn, significantly higher than of metal-free porphyrins (85-97% and 77-79%, respectively). Previously experimentally we were found that under the action cationic porphyrins and metalloporphyrins on Gram (+) and Gram (-) microorganisms efficiency of metalloporphyrins Zn-TOEt4PyP and Zn-TBut4PyP in 3-5 times was higher than the metal-free porphyrins. In this study under the action of porphyrins and their Zn-derivatives on microorganism St. aureus such an effect was confirmed. Using the LED with a peak emission of 405 nm and a power density of 70 mW/cm2, and irradiation time of microorganisms from 5 to 30 minutes we have found,

that at a concentration of 0.1 ug/ml the highest efficiency is observed of metalloporphyrin Zn-TBut3PyP. Upon irradiation of 10 and 15 minutes his efficiency is 3-5 times higher than the metal-free porphyrin TOEt4PyP, and irradiation for 30 minutes via Zn-TBut3PyP is practically completely destroys microorganisms. These data correlate with the quantum yield of singlet oxygen for photosensitizers. 30 minutes of direct sun exposure (power density of 70 mW/cm2) on solutions of photosensitizers showed that a significant photobleaching of porphyrins and metalloporphyrins does not occur. Thus, Zn-containing cationic metalloporphyrins are highly efficient photosensitizers for photodynamic inactivation of microorganisms and PDT.

8942-17, Session 4

Optical measurements of CO2 reactivity in children With single-ventricle physiology: comparison with ASL-MRI

Peter Schwab, The Children's Hospital of Philadelphia (United States); Jennifer M. Lynch, The Children's Hospital of Philadelphia (United States) and Univ. of Pennsylvania (United States); Erin M. Buckley, Harvard Medical School (United States); David Busch, Lisa Montenegro, Susan Nicolson, Daniel J. Licht, The Children's Hospital of Philadelphia (United States); Arjun G. Yodh, Univ. of Pennsylvania (United States); Mark A. Fogel, The Children's Hospital of Philadelphia (United States)

Introduction

Depressed CO2 reactivity is associated with hypoxic-ischemic neurological injury in pediatric populations. We employed Arterial Spin Labeling MRI (ASL-MRI) and Diffuse Correlation Spectroscopy (DCS) to non-invasively quantify CO2 reactivity in a population of children with congenital heart defects (CHD), and assessed the agreement between the MRI and optical techniques.

Methods

Children with CHD admitted to the Children's Hospital of Philadelphia for staged palliative surgery were recruited. Prior to surgery, simultaneous ASL-MRI and DCS measurements of baseline and hypercapnic cerebral blood flow (CBF) were obtained. CO2 reactivity was quantified as the percentage increase in CBF per mmHg increase in arterial partial pressure of CO2 during hypercapnia. Concordance correlation statistics were used to compare ASL-MRI and DCS measurements.

Results

Thirty-five patients (avg. age 1.39 [0.0-5.0]) were included in our analysis. Median CO2 reactivity was 1.09%/mmHg. Close agreement between ASL-MRI and DCS techniques was observed, with a concordance correlation coefficient of 0.99 ($R=0.757$, $p<0.001$, slope=0.67±0.21).

Discussion

The good agreement between ASL-MRI and DCS is encouraging, given that ASL is most sensitive to the diffusion of water through tissue while DCS is most sensitive to the movement of red blood cells through microvasculature. DCS is inexpensive, portable, and less intimidating for a child than MRI measurements like ASL. Longitudinal episodic optical measurements of cerebral blood flow with DCS are feasible, provide CBF data comparable to ASL data, and may be clinically useful for tracking CO2 reactivity in CHD patients as they progress through staged surgical palliation, potentially informing the timing of therapeutic interventions.

8942-18, Session 4

Anatomical co-registration using spatio-temporal features by non-contact near-infrared optical scanner

YoungJin Jung, Jean Gonzalez, Suset Rodriguez, Maximiliano Velez Mejia, Gabrielle Clark, Anuradha Godavarty, Florida International Univ. (United States)

Non-contact based near-infrared (NIR) optical imaging devices are developed for non-invasive imaging of deep tissues in various clinical applications. Most of these devices focus on obtaining the spatial information for anatomical co-registration of blood vessels as in sub-surface vein localization applications. In the current study, the anatomical co-registration of blood vessels based on spatio-temporal features was performed using NIR optical imaging without the use of external contrast agents. A 710 nm LED source and a compact CCD camera system were employed during simple cuff (0 to 60 mmHg) experiment in order to acquire the dynamic NIR data from the dorsum of a hand. The spatio-temporal features of dynamic NIR data were extracted from the cuff experimental study to localize vessels according to blood dynamics. The blood vessel shape is currently reconstructed from the dynamic data based on spatio-temporal features. Demonstrating the spatio-temporal feature of blood dynamic imaging using a portable non-contact NIR imaging device without external contrast agents is significant for applications such as peripheral vascular diseases.

8942-19, Session 4

In vivo label-free monitoring microvascular and lymphatic vessel changes and dynamics during wound healing in mouse ear pinna using optical microangiography

Siavash Yousefi, Ruikang Wang, Univ. of Washington (United States)

The circulatory network in mammals composed of cardiovascular and lymphatic system delivers oxygen, nutrition, immune cells and hormones to tissue and collects waste materials from cells. The role of circulatory system in recovering and healing wounds and generation and growth of new tissue is very essential. However, the role and response of collateral capillaries and lymphatic vessels in the healing process is not very well known. Ultrahigh-sensitive OMAG (UHS-OMAG) technology is capable of imaging microstructure and microvasculature down to capillary level. Since the lymph fluid is clear and transparent, lymphatic vessels appear as reduced scattering vessel-like areas in OCT structure cross-section images.

In this paper, we propose a lymphatic vessel segmentation technique from structure cross-section images. The segmentation is applied to three-dimensional frames acquired from a healing tissue and blood and lymphatic vessels are non-invasively segmented and visualized alongside blood vessels acquired from UHS-OMAG to demonstrate healing process in a mouse ear pinna. Lymphatic vessels are typically very small in the normal tissue while their size dramatically increases during inflammatory phase and therefore can be picked up and segmented by our system/method.

To the best of our knowledge, this is the first time that a systematic segmentation approach is presented to identify lymphatic vessels in OCT structure images. The advantage of using OCT over other image modalities is that it allows three-dimensional label-free non-invasive simultaneous imaging structure, blood vessels and lymphatics.

8942-20, Session 4

Wavelet and multifractal based analysis on DIC Images in stromal region to distinguish between normal and cancerous tissue

Sabyasachi Mukhopadhyay, Nandan Kr Das, Indian Institute of Science Education and Research Kolkata (India); Asima Pradhan, Indian Institute of Technology Kanpur (India); Nirmalya Ghosh, Prasanta K. Panigrahi, Indian Institute of Science Education and Research Kolkata (India)

Differential Interference Contrast Image (DIC) provides well-known technique to achieve high contrast image of spatial refractive index fluctuation. DIC images of cervical cancer and normal tissues were taken from stromal region, on which wavelet transform and multi-fractal analysis have been applied. Discrete wavelet transform (DWT) through Daubechies basis has been done for identifying fluctuations over polynomial trends for clear characterization and differentiation of tissues. We have carried out a systematic investigation of denoised images through the continuous Morlet wavelet. The scalogram reveals that there is a sudden change in coefficient peak values in grade 1 as compared to the normal case. The same increases with the grades upto grade 3. Wavelet Normalized energy plots are computed in order to show the difference from normal to cancerous tissues. Using the multi-fractal detrended fluctuation analysis (MFDFA), we have observed that the values of Hurst exponent and width of singularity spectrum increase as cancer progresses from healthy (normal) tissue.

8942-21, Session 5

Tissue optical clearing window for blood flow monitoring with laser speckle contrast imaging

Jing Wang, Yang Zhang, Pengcheng Li, Qingming Luo, Dan Zhu, Huazhong Univ. of Science and Technology (China)

Laser speckle contrast imaging (LSCI) can provide a two-dimensional map of blood flow at high temporal-spatial resolution, but suffer from turbid tissues, and usually has to establish windows by surgical operation. Tissue optical clearing (TOC) technique has shown significant potential for improving biomedical optical imaging. However, in vivo TOC technique needs to meet the follows: fast treating, enough transparent and safe to animals, so its development is relative lagging. Fortunately, some innovative optical clearing methods for in vivo were proposed and opened some windows on skin or skull, which enhanced the contrast and resolution of laser speckle contrast imaging (LSCI) for blood flow monitoring. Here, the current status is reviewed, which includes the principle of TOC induced improvement of LSCI, progress in tissue optical clearing windows for blood flow monitoring, the safety of optical clearing agents to animals. Up till now, various transparent skin windows and cranial window are established by topical application of optical clearing agents instead of surgery, which enable LSCI to monitor dermal or cerebral blood flow with high resolution and contrast. And safety investigations show that it is possible to repeatedly image dermal blood flow by a switchable transparent skin window without side effect. The development trendy will focus on the safety and widely applications in biomedicine.

8942-22, Session 5

Enhanced resolution and contrast of photoacoustic microscopy with optical clearing methods *(Invited Paper)*

Dan Zhu, Xiaoquan Yang, Yang Zhang, Yanyan Liu, Rui Shi, Hui Gong, qingming Luo, Huazhong Univ. of Science and Technology (China)

Combining various kinds of advantages, such as the high optical sensitivity and extremely weak acoustic scattering, Photoacoustic microscopy (PAM) can map the distribution of endogenous and exogenous absorption contrast deep in the tissue with unprecedented spatial resolution. The PAM has showed significant potential for structural, functional, and molecular imaging. However, the imaging quality of PAM is still deteriorated because the maximum penetration depth of PAM in biological tissue suffers from the strong scattering of tissues. With the development of tissue optical clearing technique, the penetration depth of light into tissue can be effectively improved. In this presentation, we demonstrate that optical clearing methods induced enhancement of the image quality of PAM by topical application on skin or skull by in vitro experiments. Furthermore, combined with skin optical clearing or skull, the PAM was used to image the subcutaneous vascular network through transparent skin or cerebrovascular through transparent skull, respectively. The results show that the resolution and contrast of vascular were improved obviously.

8942-23, Session 5

Laser-induced modification of pore structure in sclera towards a new method for intraocular pressure normalization

Olga I. Baum, Emil N. Sobol, Eugeny M. Shcherbakov, Institute on Laser and Information Technologies (Russian Federation)

A new approach to increase of uveoscleral outflow path under thermo-mechanical effect of laser radiation have been presented. Experiments have been performed with 40 minipig eyes in-vitro and with 20 rabbit eyes in-vivo using an Erbium doped glass fiber laser of 1.56 microns in wavelength.

Light Scattering and Atomic Force Microscopy measurements have been used to study alteration in scleral structure due to non-uniform laser heating. Rarefication of the collagen structure in the laser affected zone and formation of sub-micron pores have been clearly recognized.

Laser settings providing substantial alterations in light scattering correlate well with laser parameters required for significant increase of water permeability resulting in normalization of the intraocular pressure.

Substantial increase of water permeability of eye tissues can be a novel approach to normalize the intraocular pressure. The results obtained are the basis of a new effective method for glaucoma treatment.

8942-24, Session 5

Imaging of the interaction of low frequency electric fields with biological tissues with and without optical clearing by optical coherence tomography

Adrián Peña Delgado, Alexander Doronin, Igor V. Meglinski, Univ. of Otago (New Zealand)

Low frequency electric fields propagating in ex vivo biological tissues have been observed by using double correlation optical coherence

tomography (OCT) before and during the optical clearing. Optical clearing has been used as a technique to reduce tissue scattering by matching refractive index between tissue components, causing tissue dehydration and thickness reduction. The results present the direct observation of the scope of the electric field influencing biological tissues with OCT. The results show that variation in voltage and frequency of the applied electric field relates exponentially to the magnitude of its influence on biological tissue in vitro. The magnitude of influence is about twice more for fresh tissue samples in comparison to non-fresh ones. In addition, this study has partially been focused on the evaluation of the thickness reduction in tissues during the application of optical clearing agent. The obtained results suggest that OCT can be used for observation and quantitative evaluation of the electro-kinetic changes in biological tissues under different physiological conditions.

8942-25, Session 7

In vivo microcirculation imaging of reactive hyperaemia in human finger ischemia using correlation mapping optical coherence tomography

Haroon Zafar, National Univ. of Ireland, Galway (Ireland); Martin J. Leahy, National Univ. of Ireland, Galway (Ireland) and National Biophotonics and Imaging Platform (Ireland)

In this work correlation mapping optical coherence tomography (cmOCT) technique was used for in vivo microcirculation imaging of reactive hyperemia in human finger ischemia. The cmOCT technique developed by our group uses standard OCT image acquisition and a novel software post-processing protocol based on correlation statistics to generate three dimensional (3D) microcirculation structure maps in a completely non-contact and non-invasive manner. The cmOCT technique extracts the flow information from the OCT data sets using purely the OCT signal intensity without the requirement of phase information. All the OCT imaging in this work has been performed using frequency domain OCT system which acquires a 3D volume of 1024x1024 A-scans, each of 512 pixels deep in approximately 70 s. The OCT B-scans of the human finger before, during and after the brief period of ischemia were acquired over an area of 3x3mm. The resulting OCT volume of B-scans in each case was processed using the cmOCT technique with a 7x7 kernel in 116 s to generate 3D microcirculation structure maps. The correlation gradient has been used to identify related micro channels and how the associated vessels relate to each other.

8942-26, Session 7

Simultaneous measurement of flow and diffusion using optical coherence tomography

Nicolas Weiss, Ton G. van Leeuwen, Academisch Medisch Ctr. (Netherlands); Jeroen Kalkman, Academisch Medisch Ctr. (Netherlands) and Technische Univ. Delft (Netherlands)

Tissue perfusion is a key functional parameter used to describe the supply of blood to tissue. The total supply of blood can be measured in the arteries connected to the tissue. However, the arteries do not give any information about the local delivery of blood. Local delivery of blood takes place in the microvasculature where blood delivers oxygen and removes metabolic waste products. Monitoring of dynamic processes such as diffusion and flow in the microcirculation is a good indicator of the state of tissue perfusion.

Optical coherence tomography (OCT) is an imaging technique in which low coherence interferometry is used to produce depth resolved complex-valued backscatter profiles of (biological) samples up to a few milliliters deep [1]. Several studies have shown the potential of OCT to measure sample dynamics, such as, longitudinal flow [2] and particle diffusion [3,4].

In principle, the OCT correlation function allows for simultaneous determination of flow and diffusion [5]. Here, we present a model for the OCT correlation function to measure the longitudinal and transverse flow velocities and the diffusion coefficient in colloidal suspensions. We demonstrate on a flow phantom simultaneous imaging of sample morphology, flow, and diffusion at micrometer scale in a single measurement. We anticipate that the proposed method opens up new opportunities for the study of complex rheological systems, such as, blood dynamics in the microvasculature.

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8942-27, Session 7

Automated choroidal segmentation method in human eye with 1050nm optical coherence tomography

Cindy Liu, The Harker School (United States); Ruikang Wang, Univ. of Washington (United States)

Choroidal thickness (ChT), defined as the distance between the retinal pigment epithelium (RPE) and the choroid-sclera interface (CSI), is highly correlated with various ocular disorders like high myopia, diabetic retinopathy, central serous chorioretinopathy, etc. Long wavelength OCT in 1050 nm has the ability to penetrate deep to the CSI, making the measurement of ChT possible. An ability to accurately segment CSI and RPE would be important in extracting clinical information from OCT images. However, automated CSI segmentation is challenging due to a weak boundary in the lower choroid and inconsistent texture with varied blood vessels. To meet this challenge, we propose an automated method based on the k-means clustering algorithm. The method is effective in segmenting the CSI and RPE, making the calculation of the ChT thickness quick and accurate. The performance of the method was tested by 422 frames from 3 subjects without posterior disorders. The processing time was about 0.3 seconds per frame and the average time was around 0.5 seconds per frame with correction among frames, which is faster than reported algorithms. We show that the method is more accurate and consistent with less root-mean-square error (RMSE) (1.4 μm for RPE and 7.5 μm for CSI) than manual segmentation. Further investigation will include optimizing the algorithm to cover more OCT images captured from patients, and increasing the speed and robustness of the segmentation method.

8942-28, Session 7

Temporal dynamics of cuttlefish (Sepia bandensis) camouflage

Ryan M. Nolan, Mohammad Jaber, Darold R. Spillman Jr., Eric J. Chaney, Guillermo L. Monroy, Andrew J. Bower, Nathan D. Shemonski, Joanne Li, Ryan L. Shelton, Marina Marjanovic, Univ. of Illinois at Urbana-Champaign (United States); John M. Cwaygel, Sailfin Pet Shop, Inc. (United States); Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Cuttlefish are commonly referred to as “chameleons of the sea” for their ability to rapidly and dynamically alter their skin color, pattern, and texture for camouflage or communication. The sophisticated mechanism behind this ability is due to the dense distribution of pigmented chromatophore cells and structural reflector iridophore and leucophore cells in the skin, and also the ability to individually control each cell. Chromatophores actively reflect and/or transmit shades of black, brown, yellow, orange, and red when the pigment-filled sac is expanded by radial muscle fibers, but when relaxed, these structures retract until barely visible. Iridophores and leucophores, on the other hand, are responsible for all other colors, but are dependent on ambient light reflectance. While marine biologists have regularly studied these cephalopods, few studies have investigated their real-time temporal dynamics.

We have optically stimulated over 14 captive-hatched and raised, mature Sepia bandensis cuttlefish to assess the temporal dynamics of individual chromatophore size and intensity distribution modulation in adaptive coloration. Individually isolated cuttlefish were imaged with high-speed, high-resolution cameras and a high-resolution submersible spectrometer probe to quantify optical property and pattern fluctuations in the skin under multiple source and/or substrate stimulation. By correlating quantitative temporal and spectral dynamics of chromatophores with optical coherence tomography, multiphoton microscopy, and histology, we can better characterize and decipher the complex coordination of the different chromatophore colors used during adaptive camouflage or coloration. Elucidating these mechanisms and their neurobiological control offers the potential for new biomimetic strategies for advanced optical displays and dynamic patterned surfaces.

8942-29, Session 7

Application of OCM techniques to the EMT study in an embryonic chick heart

Siyu Ma, Rui Wang, Clemson Univ. (United States); Raymond B. Runyan, The Univ. of Arizona (United States); Richard L. Goodwin, Univ. of South Carolina School of Medicine (United States); Roger R. Markwald, Thomas K. Borg, Medical Univ. of South Carolina (United States); Bruce Z. Gao, Clemson Univ. (United States)

Optical coherence microscopy (OCM) is a non-invasive technique in imaging tissues and cells within highly scattering medium. It combines low coherence interferometry with confocal microscopy to perform sectioning imaging beneath the surface of a sample within one millimeter and rejects out of focus light to provide images with submicron lateral resolution. OCM outmaneuvers confocal imaging performance in both penetration depth and sensitivity. However, OCM received little attention due to the overlap of its application with confocal microscopy and the worse lateral resolution and signal to noise ratio than those provided by a confocal. In this paper, we report the unique application of OCM in the in vivo investigation of the endothelial cell to mesenchymal cell transition (EMT). Heart valve malformation is a common congenital heart defect, which has recently been hypothesized to be related to the cilia’s improper gene expression in response to the high shear stress created by the blood flow; this results in the failure of EMT and ultimately leading to remodeling of heart morphology and alternation of hemodynamic conditions. A noninvasive imaging technique capable of penetrating the heart tube and performing cellular level imaging is critical to the in vivo study of EMT. Our results demonstrate that OCM is suitable to visualize the EMT in the heart tube of an embryonic chick. With this technique, further studies of how the interactions of the genetic and environmental factors affect embryonic heart remodeling become achievable.

8942-30, Session 7

Functional optical imaging of oxygen supply and demand *(Invited Paper)*

Melissa C. Skala, Vanderbilt Univ. (United States)

No Abstract Available

8942-31, Session 8

Volumetric mass flux density measurement using super-resolution optical coherence microangiography

Siavash Yousefi, Ruikang Wang, Univ. of Washington (United States)

Measuring total blood flow passing through arteries/veins is clinically valuable in diagnosis and monitoring microvasculature-related disease in biological tissues. Traditionally by utilizing Doppler optical coherence tomography (DOCT), total blood flow is estimated by measuring blood flow velocity in the desired vessels and integrating over the vessel area. Recent studies in the literature show feasibility and application of DOCT in measuring total blood flow in human/rodent retina and rodent brain. Using conventional DOCT, blood cell concentration is assumed to be constant while this is not true and some variations exist. Basically, it is completely ignored that the DOCT measurement is based on backscattering from (typically) red blood cells while the final value is measuring blood volume (rather than content). Another limitation of DOCT is that the resolution of the DOCT technique is limited to the number of temporal measurements at the same location, which may increase the scanning time and data storage size. We propose a super-resolution technique based on multiple signal classification (MUSIC) algorithm to estimate volumetric mass flux density of blood to estimate cell concentration and velocity at the same time. Due to its super-resolution nature, the resolution is not limited to the number of temporal scans. For the proof of concept, the algorithm is tested on a tissue-mimicking flow phantom.

8942-32, Session 8

Large field of view and depth specific cortical microvascular imaging underlies regional differences in ischemic brain *(Invited Paper)*

Jia Qin, Lei Shi, Suzan Dziennis, Ruikang Wang, Univ. of Washington (United States)

No imaging technique is available that can satisfactorily extract blood flow, blood vessel morphology, oxygenation and tissue morphology from in vivo microcirculatory tissue beds, with large field of view and sufficient resolution at defined depth without any harm to the tissue. In order for more effective therapeutics, we need to determine the occasion and position of penumbra, the area of brain that is damaged but not yet dead after focal ischemia. Here we develop an integrated multi-functional imaging system, in which SDW-LSI (synchronized dual wavelength laser speckle imaging) is used as a guiding tool for OMAG (optical microangiography) to investigate the fine detail of tissue hemodynamics, such as vessel flow, profile, and flow direction. For 90 min brain ischemia, onsite and 24 hours following reperfusion, we use SDW-LSI to determine distinct flow and oxygenation variations for differentiation of the infarct, penumbra, reduced flow and contralateral regions. The blood volumes and typical vessel flows are quantifiable and distinct in afore mentioned regions. Variations in the blood volumes are correlated well with those in blood flow and can be coregistered with the variation of total hemoglobin concentration. Furthermore, we characterize the roles of different

vascular compartments in generating and controlling the hemodynamic response, such as the distal downstream arterioles show reversals in flow, which maybe important to mitigate the effects of vessel obstruction. These achievements may ultimately facilitate clinical diagnosis, monitoring and therapeutic interventions of neurovascular diseases, such as ischemic stroke.

8942-33, Session 8

Multi parametric imaging of cerebral hemodynamic and metabolic response followed by ischemic injury

Jia Qin, Lei Shi, Lin An, Suzan Dziennis, Ruikang Wang, Univ. of Washington (United States)

We use rodent parietal cortex as a model system and utilize a synchronized dual wavelength laser speckle imaging (SDW-LSI) technique to explore the hemodynamic response of infarct and penumbra to a brain injury (middle cerebral artery occlusion (MCAO) model). The SDW-LSI system is able to take snapshots rapidly (maximum 500 Hz) over the entire brain surface, providing key information about the hemodynamic response, in terms of which it may be used to elucidate evolution of penumbra region from onsite to 90 min of MCAO. Changes in flow are quantified as to the flow experiencing physical occlusions of the MCA normalized to that of baseline. Furthermore, the system is capable of providing information as to the changes of the concentration of oxygenated, (HbO) deoxygenated (Hb), and total hemoglobin (HbT) in the cortex based on the spectral characteristics of HbO and Hb. We observe that the oxygenation variations in the four regions are detectable and distinct. Combining the useful information, four regions of interest (ROI), infarct, penumbra, reduced flow and contralateral portions in the brain upon ischemic injury may be differentiated. Implications of our results are discussed with respect to current understanding of the mechanisms underlying MCAO. We anticipate that SDW-LSI holds promise for rapid and large field of view localization of ischemic injury.

8942-34, Session 9

Cell dynamics for contrast and diagnosis in digital holography *(Invited Paper)*

Adam Wax, Duke Univ. (United States)

No Abstract Available

8942-35, Session 9

Role of cellular adhesions in tissue dynamics spectroscopy

Daniel Merrill, Ran An, John Turek, David Nolte, Purdue Univ. (United States)

Cellular adhesions play a critical role in cell behavior, and modified expression of cellular adhesion compounds has been linked to various cancers [Makrilia, Kollias, Manolopoulos, et. al., Cancer Invest. 27:1023, 2009]. We tested the role of cellular adhesions in drug response by studying two cellular culture models: three-dimensional tumor spheroids with well-developed cellular adhesions and extracellular matrix versus dilute three-dimensional cell suspensions in agarose lacking all adhesions. Our technique for measuring the drug response for the spheroids was dynamic light scattering (DLS) in the form of biodynamic imaging, and for the suspensions was quasi-elastic light scattering (QELS). We tested several cytoskeletal chemotherapeutic drugs (nocodazole, cytochalasin-D, paclitaxel, and colchicine) on three cancer cell lines chosen from human colon adenocarcinoma (HT-29), human pancreatic adenocarcinoma (PACA2), and rat osteosarcoma (UMR106) to exhibit differences in adhesion strength. Tissue dynamics spectroscopy [Nolte, An, Turek, et al., J. Biomed. Opt. 16:087004, 2011] observed shifts in the spectral behavior of the motion of the culture samples. Comparing tumor spheroid behavior to that of cell suspensions showed shifts in the spectral motion of the cancer tissues that match predictions based on different degrees of cell-cell contacts. The HT-29 cell line, which has the strongest adhesions in the spheroid model, exhibits anomalous behavior in some cases. These results highlight the importance of using three-dimensional tissue models in drug screening with cellular adhesions being a contributory factor in phenotypic differences between the drug responses of tissue and cells.

8942-36, Session 9

Imaging of electro-kinetic properties of tissue using the amplitude and the phase of optical coherence tomography *(Invited Paper)*

Vladislav Toronov, Yuan Xu, Victor Yang, Ryerson Univ. (Canada)

The electric field induced optical changes (EIOC) measured by the optical coherence tomography (OCT) reflect the local electro-kinetic properties of the tissue. EIOC imaging can potentially be used for cancer detection. In this study we developed a method to use the amplitude and the phase of the complex OCT images to map EIOC in tissue samples. Switching the polarity of the electric field induced significant reversible changes in both the amplitude and phase of the complex OCT images. Since the images were degraded by the noise, we developed an advanced signal processing algorithm to obtain the EIOC images with reduced noise. We also developed a theoretical model explaining differences between the amplitude and phase EIOC images.

8942-7, Session PSun

Monte Carlo simulation on the effect of contact pressure on in vivo NIRS measurement

Jingying Jiang, Junsheng Lu, Tianjin Univ. (China); Hao Zhang, Tianjin University (China); Xuzheng Rong, Tianjin Univ (China); Zhenhe Ma, Northeastern University (China); Kexin Xu, Tianjin Univ. (China)

NIRS analysis is considered to be a promising noninvasive detection technique. Existing research results show that optical properties of the human skin tissue will change with different contact pressures when contact survey mode is used for in vivo NIRS measurement. The impact of the contact pressure might be greater than the impact of glucose concentration on the spectral data of NIRS. The uncertainty caused by pressure in vivo, makes it extremely difficult to get the high SNR-spectrum. In this talk, the Monte Carlo simulation has been carried on under the condition of different contact pressure. Simulation results show that the diffused reflectance and transmittance increase with rising contact pressure. Moreover, the simulation results show that the effects of contact pressure are larger than the effects of glucose concentration. Therefore it is promising to make comprehensive utilization of diffused reflectance and transmittance to eliminate the interference which caused by contact pressure.

8942-37, Session PSun

Ear feature region detection based on a combined image segmentation algorithm-KRM

Jingying Jiang, Hao Zhang, Tianjin Univ. (China); Qi Zhang, Tianjin Univ (China); Junsheng Lu, Tianjin Univ. (China); Zhenhe Ma, Northeastern University at Qinhuangdao (China); Kexin Xu, Tianjin Univ. (China)

Scale Invariant Feature Transform?SIFT?algorithm is widely used for ear feature matching and recognition. However, the application of the algorithm is usually interfered by the non-target areas within the whole image, and the interference would then affect the matching and recognition of ear features. To solve this problem, a combined image segmentation algorithm i.e. KRM was introduced in this paper, As the human ear recognition pretreatment method. Firstly, the target areas of ears were extracted by the KRM algorithm and then SIFT algorithm could be applied to the detection and matching of features. The present KRM algorithm follows three steps: (1)the image was preliminarily segmented into foreground target area and background area by using K-means clustering algorithm; (2)Region growing method was used to merge the over-segmented areas; (3)Morphology erosion filtering method was applied to obtain the final segmented regions. The experiment results showed that the KRM method could effectively improve the accuracy and robustness of ear feature matching and recognition based on SIFT algorithm.

8942-38, Session PSun

Reflectance spectroscopy for evaluating hair follicle cycle

Caihua Liu, Britton Chance Ctr. for Biomedical Photonics (China); Gauan Yue, Jianru Wang, Dan Zhu, Huazhong Univ. of Science and Technology (China)

Hair follicle, as a mini-organ with perpetually cycling of telogen, anagen and catagen, provides a valuable experimental model for studying organ regeneration. The transition of hair follicle from telogen to anagen is a significant sign for successful regeneration. So far distinguish of the hair follicle stage is mostly based on canonical histological examination and empirical speculation based on skin color. Hardly a method has been proposed to quantitatively distinguish the hair follicle stage. In this work, a commercial optical fiber spectrometer was applied to measure diffuse reflectance of mouse skin, and then the melanin volume fraction was extracted. Meanwhile, an infrared thermography was used to monitor the temperature distribution of skin surface. Histological examination was also applied to verify the hair follicle stage. By comparing the histological examination with optical measurements, it can be found that the skin diffuse reflectance was relatively high for mouse with telogen hair follicles, and decreased once hair follicles transited to anagen stage, and then increased again at catagen stage. The melanin content of skin is relatively low at telogen stage, then it increased up to the peak at anagen stage, afterwards it gradually decreased. Besides, the skin temperature was found to be higher at anagen stage than at telogen stage. This study provided a new method to quantitatively distinguish the hair follicle stage, and should be valuable for the basic and therapeutic investigations on hair regeneration.

Conference 8943:
Photons Plus Ultrasound: Imaging and Sensing 2014

Sunday - Wednesday 2 –5 February 2014

Part of Proceedings of SPIE Vol. 8943 Photons Plus Ultrasound: Imaging and Sensing 2014

8943-1, Session 1

Light-enhanced transesophageal echocardiography for evaluating central hemodynamics: towards a clinical prototype

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Adequate assessment of central hemodynamics is critical for proper diagnosis and management of cardiac diseases. For example, mixed venous oxygen saturation (SvO2), measured from pulmonary arteries, is a key indicator of the dynamic balance between the body's global oxygen supply and consumption. A low SvO2 (< 60%) is an effective prognostic marker of mortality and morbidity. Goal-oriented hemodynamic therapy targeted at a normal SvO2 could improve patient outcomes. Traditionally, central hemodynamic parameters are measured with pulmonary artery catheters (PAC). However, the intravascular insertion of PAC is a risky invasive procedure, which is associated with a 10% complication rate.

We are developing a light-enhanced transesophageal echocardiography (leTEE), which could evaluate central hemodynamic status through the esophageal wall in a non-invasive, continuous and comprehensive manner. leTEE integrates transesophageal echocardiography with photoacoustic oximetry. In the ultrasonic mode, leTEE provides real-time visualization of cardiac morphologies, and estimates cardiac output and blood pressure. Simultaneously, leTEE measures light-generated photoacoustic signals from blood, and quantifies local blood oxygenation of various cardiovascular structures. Using a bench-top system, we demonstrated leTEE could accurately measure SvO2 (bias < 1%, precision < 3%) in an en-bloc swine heart model. In addition, we will report our recent advancements towards a clinical prototype of leTEE.

8943-2, Session 1

In vivo imaging of human microcirculation with linear-array based photoacoustic tomography: a feasibility study for clinical application

Hrebesh M. Subhash, Sergey A. Alexandrov, Haroon Zafar, National Univ. of Ireland, Galway (Ireland); Jithin Jose, VisualSonics B.V. (Netherlands); Martin J. Leahy, National Univ. of Ireland, Galway (Ireland)

Recently, there has been an enormous interest in the development of photoacoustic imaging (PAI) method for clinical imaging applications such as management of cancer, including screening, diagnosis, treatment planning, and therapy monitoring, and accurate measurement of metabolic rate during early diagnosis and treatment of various skin and subcutaneous tissue disorders. A variety of PA imaging systems has been developed based on various scanning configurations and reconstruction algorithms. PA imaging system based on spherical and cylindrical detection geometry can provide large angular aperture for an accurate image reconstruction, however, they are not well suited for imaging subsurface skin features for clinical imaging application. Moreover, commonly used single element PA imaging system cannot satisfy the requirement of real-time data acquisition and imaging, which is a prerequisite for clinical scenario. PA imaging based on multi-element linear transducer array combined with multichannel collecting system is an alternative option particularly for clinical imaging of skin and subcutaneous morphologies. However, there is only limited empirical studies has been carried out in this field. In this study we demonstrate the feasibility of PA imaging system based on multi-element linear transducer for functional and metabolic imaging human skin in vivo.

8943-3, Session 1

Characterization of myocardial ablation lesions using multi-wavelength photoacoustic imaging

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Introduction:

Radiofrequency (RF) ablation to treat cardiac arrhythmia is limited by an inability to reliably assess lesion durability and transmurality in real-time. This is a preliminary study to permit thermographic and spectroscopic photoacoustic (PA) imaging, concurrent with ablation, to visualize lesion formation based on unique differences in the optical absorption spectra between normal and ablated myocardial tissue.

Methods:

Tissue samples were excised from the ventricles of fresh porcine hearts. Lesions were generated using an RF catheter ablation system (Biosense Webster Inc.) using 30 W of power, applied for 30 s. Using a combined US and PA imaging system (Verasonics Inc.), samples with lesions were imaged during ablation at three (740, 750 and 760 nm) wavelengths. The measured PA spectra were then correlated to the absorption spectra of deoxy-hemoglobin and ablated tissue to produce spectroscopic PA (sPA) images used to identify the RF lesion and to assess lesion dimensions. Tissue samples were then stained and photographed for gross pathology validation. Lesion location within the sample was determined by rigid co-registration of PA and US data with gross pathology photographs.

Results:

The location and volume of the lesion determined by sPA imaging correlated well with stained gross pathology and were within 0.1 mm and 70%, respectively.

Conclusion:

These results demonstrate that sPA imaging has potential to accurately assess RF ablation lesion size and position in clinical practice.

8943-5, Session 1

Quantification of photoacoustic microscopy images for ovarian cancer detection

Tianheng Wang, Yi Yang, Umar S. Alqasemi, Patrick D. Kumavor, Univ. of Connecticut (United States); Xiaohong Wang, Melinda

Sanders, Molly Brewer, Univ. of Connecticut Health Ctr. (United States); Quing Zhu, Univ. of Connecticut (United States)

In this paper, human ovarian tissue with malignant and benign features was imaged ex vivo using an optical-resolution photoacoustic microscopy (OR-PAM) system. The feasibility of PAM to differentiate malignant from normal ovarian tissues was explored by comparing the PAM images morphologically. Based on the observed differences between PAM images of normal and malignant ovarian tissues in microvasculature features and distributions, eight features were quantitatively extracted from the PAM images, and a logistic model was used to classify ovaries as normal or malignant. 106 PAM images from 18 ovaries were studied. 57 images were used to train the eight-parameter logistic model, and a specificity of 92.1% and a sensitivity of 84.2% were achieved; 49 images were then tested, and a specificity of 71.9% and a sensitivity of 100% were achieved. These preliminary results demonstrate the feasibility of our PAM system in mapping microvasculature networks as well as characterizing the ovarian tissue, and could be extremely valuable in assisting surgeons for in vivo evaluation of ovarian tissue during minimally invasive surgery.

8943-6, Session 1

Feasibility of transcranial photoacoustic imaging for interventional guidance of endonasal surgeries

Muyinatu A. Lediju Bell, Johns Hopkins Univ. (United States); Anastasia K. Ostrowski, Johns Hopkins Univ. (United States) and The Univ. of Michigan (United States); Peter Kazanzides, Johns Hopkins Univ. (United States); Emad M. Boctor, Johns Hopkins Outpatient Ctr. (United States)

Endonasal surgeries to remove pituitary tumors incur the deadly risk of carotid artery injury due to limitations with real-time visualization of blood vessels surrounded by bone. We propose to overcome current limitations with photoacoustic imaging. Blood vessels and surrounding bone would be illuminated by an optical fiber attached to the endonasal drill, while a transducer placed on the pterional region outside of the skull acquires images. To investigate feasibility, a plastisol phantom embedded with a spherical metal target was submerged in a water tank. The target was aligned with a 1-mm optical fiber coupled to a 1064nm Nd:YAG laser. An Ultrasonix L14-5W/60 linear transducer, placed approximately 1 cm above the phantom, acquired photoacoustic and ultrasound images of the target in the presence and absence of 2-, 4-, and 7-mm-thick human adult cadaveric skull specimens. Though visualized at 18 mm depth when no bone was present, the target was not detectable in ultrasound images when the 4- and 7-mm thick skull specimens were placed between the transducer and phantom. In contrast, the target was visible in photoacoustic images at depths of 18, 17, and 16 mm, corresponding to the placement of no skull, 4-mm-, and 7-mm-thick skull specimens, respectively. These results agree with theoretical and simulation predictions. To mimic a clinical scenario where bone reduces optical transmission prior to endonasal drill penetration through the bone, the 2-mm-thick specimen was placed between the phantom and optical fiber, while the 4-mm specimen remained between the phantom and transducer. In this case, the target was present at depths of 15-17 mm for energies ranging 9-18 mJ, with photoacoustic signal-to-noise ratios ranging 15-18 dB. Results are promising for photoacoustic-guided endonasal surgeries.

8943-98, Session 1

Thermoacoustic imaging of prostate cancer: comparison to histology

Sarah K. Patch, Stephanie Griep, Univ. of Wisconsin-Milwaukee (United States); Kenneth Jacobsohn, William A. See, Medical College of Wisconsin (United States); David Hull, Bostwick Labs. (United States)

Diagnostic imaging of prostate cancer (PCa) is notoriously difficult. To determine whether the thermoacoustic (TA) contrast mechanism could meet this unmet need, we imaged fresh human prostate specimens ex vivo and compared to the gold standard, histology. Specimens were scanned immediately after radical prostatectomy, provided as part of normal care provided to PCa patients.

Electromagnetic pulses with carrier frequency 108 MHz ensures excellent EM depth penetration. 700 ns pulses propagate 20-25 mJ into a benchtop imaging system. Tomographic spatial encoding was performed in step and shoot mode, with 1.8 degree rotational stepsizes and a 4 cm rotation radius. 2.25 MHz focused single element transducers (Olympus V2.25) received the thermoacoustic pulses, which were amplified by 54 dB and signal averaged 64 times before recording to disc.

Reconstructions performed via filtered backprojection revealed a few common features: the verumontanum is often visualized and the anterior is typically bright, due to normal healthy vasculature.

Histology slides have been digitized for comparison to select cases. Results in the few cases analysed to date include signal suppression associated with foci of large, cystically dilated acini and signal enhancement in the posterior region associated with PCa.

If reconstructions from complete tomographic data support the hypothesis that the TA contrast mechanism is sensitive to PCa, then additional work on limited angle reconstruction and use in conjunction with other imaging techniques, such as shear wave elastography, will be warranted.

8943-7, Session 2

Integrated intravascular ultrasound and optical-resolution photoacoustic microscopy with a 1-mm-diameter catheter

Xiaosong Bai, Xiao-jing Gong, Riqiang Lin, Shenzhen Institute of Advanced Technology (China); William Hau, Li Ka Shing Faculty of Medicine, University of Hong Kong (Hong Kong, China); Liang Song, Shenzhen Institute of Advanced Technology (China)

Intravascular ultrasound (IVUS) plays a vital role in assessing the severity of atherosclerosis and has greatly enriched our knowledge on atherosclerotic plaques. However, it mainly reveals the structural information of plaques. In contrast, spectroscopic and molecular photoacoustic imaging can potentially improve plaque composition identification, inflammation detection, and ultimately the stratification of plaque vulnerability and risk. In this work, we developed an integrated intravascular ultrasound and optical-resolution photoacoustic microscopy (IVUS-PAM) system with a single catheter as small as 1 mm in diameter, comparable to that of existing clinical IVUS catheters. In addition, by using a GRIN lens to focus the excitation laser pulse, the system provides an optical-diffraction limited photoacoustic lateral resolution as fine as 19.6 micrometers, ~10-fold finer than that of conventional intravascular photoacoustic imaging and existing IVUS technology. The system employs a custom-made miniaturized single-element ultrasonic transducer with a dimension of ~0.5 mm, a centre frequency of ~40 MHz, and a fractional bandwidth of ~60%. The IVUS-PAM can simultaneously acquire co-registered IVUS images with an axial resolution of ~40 micrometers and a lateral resolution of ~200 micrometers. In the future, IVUS-PAM may open up new opportunities for improved high-resolution vulnerable plaque imaging and image-guided stent deployment.

8943-8, Session 2

Intracellular temperature mapping with fluorescence-assisted photoacoustic thermometry

Liang S. Gao, Chi Zhang, Chiye Li, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Intracellular temperature sensing is an essential technique for understanding cellular thermodynamic behavior during events such as division, gene expression, and enzyme reaction. Although cellular thermometry has been realized at the single-cell level by employing tools such as micro- or nano-scale thermocouples and fluorescence nanoparticles or nanogels, most of these techniques have treated a cell as a whole and measured its average temperature. Knowledge of the average cellular temperature is insufficient for exploring thermogenesis and thermal dynamics at the level of subcellular structures.

The difficulty of achieving intracellular temperature mapping lies in a fact that it requires measuring a physical quantity sensitive to local temperature changes but independent of the sensor's concentration and excitation strength. To our knowledge, only two fluorescence-based techniques have realized intracellular temperature mapping, utilizing fluorescence lifetime and polarization anisotropy, respectively. Despite the high spatial and temperature resolution, they have accomplished in cellular imaging experiments, both methods rely on custom-developed fluorescent biosensors, limiting their accessibility to only a few laboratories.

A major impetus towards the widespread application of fluorescence microscopy is the ongoing development of fluorescent probes, which display excellent selective labeling of cellular structures. However, most commercially available fluorescent probes were not intended to be temperature sensitive. To expand the toolbox of intracellular temperature mapping technique and make it accessible to a much broader biological research community, Here, we present fluorescence-assisted photoacoustic thermometry (FAPT) for studying the intracellular thermodynamics. Compared to previous fluorescence-based methods, FAPT features the unique capability of transforming a regular fluorescence dye into a concentration- and excitation-independent temperature sensor, a fact that opens up the possibility of utilizing a large collection of commercially available fluorescent probes for intracellular temperature sensing applications.

8943-9, Session 2

Optical resolution photoacoustic imaging by the coherent control of light in a multimode fiber

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Multimode optical fibers have recently been suggested and investigated as a new promising paradigm of miniature endoscopic devices. Moreover, Optical Resolution Photoacoustic Microscopy (OR-PAM) is a rapidly developing multimodal imaging technique that combines optical excitation and acoustic detection to deliver high resolution images (on the order of micrometers) based on the optical absorption characteristics of the interrogated sample.

In this paper we present an OR-PAM implementation, where a focused spot is generated and scanned at the distal tip of a multimode optical fiber using Digital Phase Conjugation. The small diameter of multimode fibers can enable the use of ultrathin minimally invasive passive endoscopes that can render high-resolution OR-PAM images deeper than the ballistic range of light propagation in tissue. We show images of a knot of 30um diameter absorptive nylon wires with a resolution smaller than 1.5um over a 201 by 201 um field of view and we compare it against other imaging modalities.

The lack of any lenses or mechanical actuators for the focusing and scanning along with the small diameter of the fiber means that the whole rigid endoscope head can be made very small and in principle smaller than 500?m. This enables the generation of needle-type devices, in which the optical excitation is brought against the interrogated sample by directly penetrating through the tissue allowing for OR-PAM far deeper than the ballistic range of optical propagation in biological tissue.

8943-10, Session 2

Prototype study on a miniaturized dual-modality imaging system for photoacoustic microscopy and confocal fluorescence microscopy

Sung-Liang Chen, Zhixing Xie, L. Jay Guo, Xueding Wang, Univ. of Michigan (United States)

The study of tumor angiogenesis and microenvironments plays an important role in cancer diagnosis. Photoacoustic microscopy (PAM) has been demonstrated as a useful tool in in vivo mapping angiogenic microvasculature. On the other hand, fluorescence imaging has been applied to assessment of tissue anatomical structures. Besides, dual-modality systems combining PAM and confocal fluorescence microscopy (CFM) have been studied to present more comprehensive information for cancer diagnosis. Currently, the dual-modality systems with PAM and CFM are built mostly based on bulky components, highly restricting their endoscopic applications such as in vivo transurethral and transrectal imaging. Development of compact scanning heads capable of PAM and CFM modalities is still challenging and has not been realized. In this work, we designed and built a fiber-optic based PAM and CFM dual-modality imaging system using miniature components. To explore the feasibility of this device for future endoscopic applications, a microelectromechanical systems (MEMS) scanner, a miniature objective lens, and a small size optical microring resonator as an acoustic detector were employed trying to meet the requirements of miniaturization. Both the lateral resolutions of PAM and CFM were quantified to be 8.8 μm. Axial resolutions of PAM and CFM were measured to be 19 μm and 53 μm, respectively. The experiments on ex vivo animal bladder tissues demonstrate the good performance of this system in imaging not only microvasculature but also cellular structure, suggesting that this novel imaging technique holds potential for improved diagnosis and guided treatment of diseases such as bladder cancer.

8943-11, Session 2

Ultra-miniature fiber optic photoacoustic imaging probes for endoscopic applications

Edward Z. Zhang, Adrien E. Desjardins, Paul C. Beard, Univ. College London (United Kingdom)

Miniature photoacoustic probes are required for a number of important clinical applications in which the target tissue can only be accessed by introducing an endoscopic probe percutaneously or through a natural orifice. Among these applications are the assessment of cancers in the oesophagus, colon or, prostate and the guidance of interventional procedures such as needle biopsies. The design of a photoacoustic probe for endoscopic applications poses several challenges using conventional piezoelectric based receivers. These include the question of how to integrate the delivery optical fibre with the ultrasound receiver

to avoid obscuring the excitation laser pulses, obtaining the necessary level of miniaturisation and achieving low unit cost for single use applications. To address these challenges, a range of miniature all-optical laser micromachined photoacoustic probes which employ a transparent Fabry Perot ultrasound sensor at the tip of an optical fibre have been developed. This approach offers unprecedented levels of miniaturisation with probe diameters as small as 100µm, inexpensive fabrication using vacuum deposition and laser processing techniques and the potential to be combined with other imaging modalities such as OCT and pulse-echo ultrasound. Two types of probe have been a developed. A forward viewing probe for guiding interventional procedures such as needle biopsies and a sideviewing imaging probe for visualising the interior of hollow anatomical structures such as the urinary or biliary tracts. Both probes provide sub-kPa noise equivalent pressures and an acoustic bandwidth extending to 50MHz and have been evaluated using a variety of tissue phantoms and ex vivo tissues. The unprecedented levels of miniaturisation and performance that this approach provides offers the prospect of opening up a new class of minimally invasive photoacoustic applications not available to existing methods.

8943-12, Session 2

Circulating tumor cell detection using photoacoustic spectral methods

Eric M. Strohm, Elizabeth S. L. Berndl, Michael C. Kolios, Ryerson Univ. (Canada)

A method to detect circulating tumor cells (CTCs) in blood samples using ultra-high frequency photoacoustic microscopy is presented. When frequencies over 100 MHz are used, the laser-induced ultrasound wavelength is similar to the size of a single cell. Periodically varying minima and maxima occur throughout the photoacoustic power spectrum, where the spectral spacing between minima depends on the ratio of the size to sound speed within the cell. The spectral spacing and amplitude can be used to differentiate types of cells in blood. Using a photoacoustic microscope, signals from 6 acute myeloid leukemia cells (AML, 8.6?m average diameter), 13 melanoma cells (24.4?m diameter) and 22 red blood cells (7.8?m diameter) were measured using a 532nm pulsed laser (<200nJ) and a 375 MHz transducer. A dye (trypan blue) was added to facilitate photoacoustic signals from AML cells.

The spectral spacing between minima was 85±27 MHz for melanoma cells, 176±24 MHz for AML cells, and greater than 250 MHz for erythrocytes. The photoacoustic signal amplitude normalized to the incident laser energy was 1.1±0.7 mV/nJ from melanoma cells, 0.04±0.01 mV/nJ from AML cells, and 2.7±0.5 mV/nJ from erythrocytes. Plotting the normalized signal amplitude vs. spectral spacing gives a visual representation of the cell distribution, and allows for grouping of various cell types found in blood to aid in identifying CTCs. Early detection of CTCs is crucial for decreasing cancer-related mortality rates. It is envisioned that this technique could eventually be used to detect CTCs and other blood-related abnormalities in a clinical environment.

8943-13, Session 2

All optical laser scanning photoacoustic endoscopy using glancing angle deposited Fabry-Perot etalons

Parsin Haji Reza, Jason B. Sorge, Michael J. Brett, Ronald B. Moore, Roger J. Zemp, Univ. of Alberta (Canada)

In this paper we demonstrate all optical laser scanning photoacoustic microscopy using a Glancing angle deposited Fabry- Perot etalon (GLAD-FPE). This fiber-based optical resolution imaging system with ~ 6mm diameter footprint takes advantage of two fiber arms for generating and receiving photoacoustic signals. An image guide with 30,000 single-mode fibers in a 0.8 mm diameter bundle in conjunction with a GRIN lens (1.8mm diameter) is employed in order to transfer a focused scanning spot (532 nm) and refocus it into tissue. A single mode fiber and a GRIN lens with 0.5 mm diameter are used to transfer a C-band range of wavelengths to the FPE and collect the reflected light.

GLAD is a single-step physical vapor-deposition (PVD) technique used to fabricate porous nanostructured thin films. Using titanium dioxide (TiO2) with a high refractive index (n = 2.4), the GLAD technique can be employed to fabricate samples with tailored nano-porosity, refractive index periodicities, and high Q-factor reflectance spectra. A 23 µm Parylene C layer was sandwiched between two GLAD filters in order to form the FPE. The ultrasound pressure modulated the optical thickness of the FPE and hence its reflectivity. The GLAD FPE with less than 100 Pa sensitivity was made as small as 3mm by 3mm. The FPE is in contact with the sample and it can easily be replaced and sterilized. Phantom studies indicate ~7 ?m resolution. The proposed system with a sub-mm probe footprint is very flexible and may offer a new range of possibilities for clinical applications.

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8943-14, Session 3

Direct tissue oxygen monitoring by in vivo photoacoustic lifetime imaging (PALI)

Qi Shao, Ekaterina Morgounova, Shai Asheknazi, Univ. of Minnesota (United States)

Tissue oxygen plays a critical role in maintaining tissue viability and in various diseases, including response to therapy. Images of oxygen distribution provide the history of tissue hypoxia and evidence of oxygen availability in the circulatory system. Currently available methods of direct measuring or imaging tissue oxygen all have significant limitations. Previously, we have reported a non-invasive in vivo imaging modality based on photoacoustic lifetime. The technique maps the excited triplet state of oxygen-sensitive dye, thus reflects the spatial and temporal distribution of tissue oxygen. We have applied PALI on tumor hypoxia in small animals, and the hypoxic region imaged by PALI is consistent with the site of the tumor imaged by ultrasound.

Here, we present two studies of applying PALI to monitor changes of tissue oxygen by modulations. The first study involves an acute ischemia model using a thin thread tied around the hind limb of a normal mouse to reduce the blood flow. PALI images were acquired before, during, and after the restriction. The drop of muscle pO2 and recovery from hypoxia due to reperfusion were observed by PALI tracking the same region. The second study modulates tissue oxygen by controlling the percentage of oxygen the mouse inhales. We demonstrate that PALI is able to reflect the change of oxygen level with respect to both hyperbaric and hypobaric conditions.

We expect this technique to be very attractive for a range of clinical applications in which tissue oxygen mapping would improve therapy decision making and treatment planning.

8943-15, Session 3

Photoacoustic and fluorescence imaged-guided delivery of photosensitizers using poly(ethylene glycol) covered gold nanostructures for enhanced photodynamic therapy

Mansik Jeon, Univ. at Buffalo (United States) and Pohang Univ. of Science and Technology (Korea, Republic of); Avinash Srivatsan, Roswell Park Cancer Institute (United States); Samir Jenkins, Jingyi Chen, Univ. of Arkansas (United States); Ravindra Pandey, Roswell Park Cancer Institute (United States); Chulhong Kim, Pohang Univ. of Science and Technology (Korea, Republic of) and Univ. at Buffalo (United States)

We have demonstrated a robust non-covalent method to encapsulate and deliver hydrophobic molecules in a controllable way based on poly (ethylene glycol) (PEG) monolayer covered Au nanocage (AuNC) platforms. The protein-rich in vivo environment was mimicked to study the release kinetics during delivery. To facilitate monitoring, a hydrophobic photosensitizer, 3-devinyl-3-(1'-hexyloxyethyl) pyropheophorbide (HPPH), was used for this study. The release rate was found to increase with surface charge in the order of positively charged (cationic terminus) > neutral (methoxy terminus) > negatively charged (anionic terminus) and to remain constant with molecular weight of PEG < 5,000. Using a surface cross-linking approach, the release was largely inhibited, but external stimuli (e.g. light irradiation) could enable on-demand release through the photothermal (PT) effect of the AuNCs. The HPPH release monitoring using the photoacoustic tomography shows a signal decrease as a function of release time. Further, 1O2 generation was found to be notably enhanced by the AuNC-HPPH conjugate. In this work, the use of AuNCs with LSPR in the NIR region enables the in vivo tracking of photosensitizer delivery by dual imaging modalities, PAT and fluorescence imaging. Furthermore, the efficacy of the PDT is significantly improved by the effective delivery and the electromagnetic field of the metal nanoparticles. In addition, although AuNCs were largely accumulated in livers, spleens, and kidneys of the mice in vivo, no toxicity was observed in the mice injected with the highest dose (100 nM) of AuNCs.

8943-16, Session 3

Broadening detection view of a linear-array-based photoacoustic computed tomography system using a planar acoustic reflector

Guo Li, Jun Xia, Konstantin I. Maslov, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Photoacoustic computed tomography (PACT) with a linear transducer array suffers from the limited-view problem. For instance, a linear transducer array cannot receive photoacoustic waves propagating along the lateral direction, which is orthogonal with the acoustic axis of the transducer array. Thus, in the reconstructed image, interfaces parallel to the acoustic axis disappear. To increase the two-dimensional aperture, it is possible to circularly scan the linear transducer array around the object. However, the circular scan is time consuming and therefore decreases the advantage of high-speed imaging achievable with a linear transducer array. Here we increase the detection view coverage of a high-frequency linear transducer array by using a planar acoustic reflector, placed at a 45-degree angle adjacent to the linear transducer array. The planar acoustic reflector creates a virtual linear transducer array, which is perpendicular to the physical array. The combined arrays receive photoacoustic waves along both the axial and lateral directions of the physical array, and thus double the detection angle coverage without sacrificing the imaging speed. We validated the system through phantom and animal studies. Our experimental results show that the proposed system greatly increases the detection aperture and significantly enhances the image quality of the linear-array-based PACT system.

8943-17, Session 3

Cellulose nanoparticles: photoacoustic contrast agents that biodegrade to simple sugars

Jesse V. Jokerst, Sarah Bohndiek, Sanjiv S. Gambhir M.D., Stanford Univ. (United States)

Cellulose nanoparticles (CNPs) were synthesized through acidic cleavage of cellulose linters and purified with centrifugation. TEM indicated that the nanoparticles were 132 ± 46 nm; the polydispersity index was 0.138. Ex vivo characterization showed a photoacoustic limit of detection of 0.02 mg/mL CNPs, and the photoacoustic signal of CNPs was 1.5- to 3.0-fold higher than gold nanorods (also at 700 nm resonance) on a particle-to-particle basis. Cell toxicity assays suggested that overnight doses below 0.31 mg/mL CNPs produced no significant ($p>0.05$) impact on cell metabolism. Intravenous doses up to 0.24 mg were tolerated well in nude mice. Subcutaneous and orthotopic tumor xenografts of the OV2008 ovarian cancer cell line were then created in nude mice. Data was collected with a Nexus128 scanner from Endra LifeSciences. Spectral data used a LAZR system from Visualsonics both at 700 nm excitation. We injected CNPs (0.024 mg, 0.048 mg, and 0.80 mg) via tail vein and showed that the tumor photoacoustic signal reached maximum increase between 10 and 20 minutes. All injected concentrations were statistically ($p<0.05$) elevated relative to the control group with n=3 mice in each group, and dose and signal had a linear relationship at $R2>0.96$ suggesting quantitative signal. CNP biodegradation was demonstrated ex vivo with a glucose assay. CNPs in the presence of cellulase were reduced to free glucose in under than four hours. The glucose concentration before addition of cellulase was not detectable, but increased to 92.1 µg/mL in four hours. CNPs in the absence of cellulase did not produce glucose. Small fragments of nanoparticle in the treated cohort were observed with electron microscopy. There are few photoacoustic contrast agents that offer both high signal intensity and obvious clearance/biodegradation profiles. To the best of our knowledge, this is the first example of a sugar-based photoacoustic contrast agent with important implications for clinical translation of this emerging molecular imaging modality.

8943-18, Session 3

3D laser optoacoustic ultrasonic imaging system (LOUIS-3D) for research in mice

Sergey A. Ermilov, Richard Su, André Conjusteau, TomoWave Laboratories, Inc. (United States); Fatima Anis, Mark A. Anastasio, Washington Univ. in St. Louis (United States); Alexander A. Oraevsky, TomoWave Laboratories, Inc. (United States)

In this presentation, we introduce an improved prototype of three-dimensional imaging system that combines optoacoustic tomography (OAT) and laser ultrasound tomography (LUT) to obtain coregistered maps of tissue optical absorption, speed of sound (SoS) and acoustic attenuation (AcA). The tomographic scan is performed by a 360 degree rotation of a mouse with respect to an arc-shaped array of wide band ultrasonic transducers. Broadband laser ultrasound emitters are arranged in another arc pattern and are positioned opposite and orthogonal to the array of transducers. This imaging geometry allows reconstruction of volumes that depict SoS and AcA distributions from the measured time of flight and transmission data. A Q-switched laser system is used to establish optoacoustic illumination pattern appropriate for deep tissue imaging with a tunable (730-840 nm) output wavelengths

operated at 10 Hz pulse repetition rate. A 532 nm wavelength output, being mostly absorbed within a narrow superficial layer of skin, is used to outline the visualized biological object. The object's boundary is then used in reconstruction of volumetric LUT images, which provide valuable anatomical information on tissue structures. The reconstructed LUT images can subsequently be employed by an optoacoustic reconstruction algorithm to compensate for acoustic wavefield aberration and thereby improve the accuracy of the reconstructed images of the absorbed optical energy.

8943-19, Session 3

Photoacoustic imaging and photothermolysis treatment of tumors mediated by nanoparticles

Geng Ku, Min Zhou, Shaoli Song, Qian Huang, Chun Li, The Univ. of Texas M.D. Anderson Cancer Ctr. (United States)

Photoacoustic images of female mice bearing tumors (i.e. MDA-MB231 developed from female mamary fat pads) have been successfully acquired by employing two types of nanoparticles, hollow gold nanoshells (HAuNS) and copper sulfide nanoparticles (CuS NP) targeted to cell surface receptors as optical contrast agents. Laser pulses whose wavelength are tunable around ~800 nm from Ti:Sapphire laser and 1064 nm from Q-Switched Nd:YAG , corresponding to peak absorption of HAuNS and CuSNP, are applied to excite photoacoustic signals, respectively. Photothermolysis is simultaneously induced with photoacoustic signals by the same laser pulses and can be significant to cause cell damage at higher laser energies. Photoacoustic signals are acquired by scanning ultrasonic transducer (2.25 MHz) around imaging sample illuminated with pulsed laser (~100 mJ/cm2 for duration of 5 minutes) and processed to form photoacoustic image. The photoacoustic images clearly revealed tumor structure and its associated angiogenic blood vessels, as well as functional information such as oxygen saturation and nanoparticles uptake and distribution. The laser illumination fluence of 1 J/cm2 and 5 minutes results in a significant increase of severity of histopathologic findings including necrosis and hemorrhage/edema in laser treated region. Photothermolysis experiment in cell culture shows that some A431 cells present blebbing, dendrites damage, and conceivable cell membrane damage when the cells were treated with HAuNS conjugated with C225 antibody directed at EGF receptors, followed with laser pulses at 740 nm. Our experimental data and simulation suggest that photoacoustic imaging and photothermolysis treatment mediated with nanoparticles is a promising platform for laser-based theranostic procedures.

8943-20, Session 3

Real-time optoacoustic monitoring of stroke

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Characterizing disease progression and identifying possible therapeutic interventions in stroke is greatly aided by the use of longitudinal function imaging studies. In this study, we investigate the applicability of real-time multispectral optoacoustic tomography (MSOT) as a tool for non-invasive monitoring of the progression of stroke in the whole brain. The middle cerebral artery occlusion (MCAO) method was used to induce stroke. Mice were imaged under isoflurane anesthesia preoperatively and at several time points during and after the 60-minute occlusion. The animals were sacrificed after 24 hours and their excised brains frozen at -80°C for sectioning. The cryosection were stained using H&E staining to identify the ischemic lesion. Major vessels are readily identifiable in the whole mouse head in the in vivo optoacoustic scans. During ischemia, a reduction in cerebral blood volume is detectable in the cortex. Post ischemia, spectral unmixing of the optoacoustic signals shows an asymmetry of the deoxygenated hemoglobin in the hemisphere affected by MCAO. This hypoxic area was mainly located around the boundary of the ischemic lesion and was therefore identified as the ischemic penumbra. Non-invasive functional MSOT imaging is able to visualize the hypoxic penumbra in brains affected by stroke. Since stopping the spread of the infarct area and revitalizing the penumbra is central in stroke research, MSOT imaging may prove valuable in the monitoring and development of new treatments.

8943-21, Session 4

Multispectral photoacoustic imaging with a clinical ultrasound imaging system

Daniil I. Nikitichev, Jean-Martial Mari, Rocio P. Soto-Astorga, Alexander C. Mosse, Paul C. Beard, Adrien E. Desjardins, Univ. College London (United Kingdom)

Multispectral photoacoustic imaging can provide tissue contrast that is complementary to B-mode ultrasound imaging for guiding percutaneous medical procedures such as biopsies and injections. We present a system with a clinical imaging probe that acquires multispectral photoacoustic images across the wavelength ranges of 700 to 1000 nm and 1100 to 2000 nm, with co-registered B-mode ultrasound images. To illuminate deep tissue targets, excitation light can be delivered through a single optical fibre that is integrated into a needle. Using phantoms and tissues ex vivo, it is shown that this system can provide contrast for oxy- and deoxy-haemoglobin, water, and lipids. Challenges involved with performing multispectral photoacoustic imaging using a clinical imaging scanner and a free-running ND:YAG-OPO source, including controlling image acquisition speed and acquiring excitation pulse amplitudes, are discussed. This system has strong potential to provide clinically relevant information to highlight procedural targets that are challenging to visualise with conventional methods.

8943-22, Session 4

Photoacoustic imaging of prostate brachytherapy seeds with transurethral light delivery

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We present a novel approach to photoacoustic imaging of prostate brachytherapy seeds utilizing an existing urinary catheter for transurethral light delivery. An in vivo canine prostate was surgically implanted with non-radioactive brachytherapy seeds under transrectal ultrasound guidance. The seeds were coated with black India ink to enhance optical absorption. The prostate was excised shortly after euthanasia, a urinary catheter was inserted in the urethra of the prostate, and the prostate and catheter were fixed in gelatin. A 1-mm core diameter optical fiber coupled to a 1064 nm Nd:YAG laser was inserted in the urinary catheter. The average laser energy at the tip of the fiber was varied from 6-15 mJ. An Ultrasonix SonixTouch scanner, transrectal ultrasound probe with curvilinear (BPC8-4) and linear (BPL9-5) arrays, and DAQ unit were utilized for synchronized laser light emission and photoacoustic signal acquisition. A channel was created to insert the transrectal probe in the gelatin at a similar distance from the prostate and with a similar orientation compared to the in vivo probe-prostate relationship. The implanted brachytherapy seeds were visualized at radial distances of 13-16 mm from the catheter. Multiple brachytherapy seeds were

simultaneously visualized with each array of the transrectal probe. Mean contrast and signal-to-noise ratios ranged 1.2-7.0 dB and 8-13 dB, respectively, with delay-and-sum beamforming and 25-37dB and 10-16dB, respectively, with short-lag spatial coherence (SLSC) beamforming applied to the received photoacoustic data. This work is the first to demonstrate the feasibility of photoacoustic imaging of prostate brachytherapy seeds using a transurethral light delivery method.

8943-23, Session 4

Multi-modal acousto-optic/echography imaging of ex-vivo liver tumors at 800 nm by wavefront adaptive holography

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Biological tissues are very strong light-scattering media. As a consequence, current medical imaging devices do not allow optical imaging of thick samples unless invasive techniques are used. Acousto-optic imaging is a light-ultrasound coupling technique that uses the sidebands created by acousto-optic effect to map the local light intensity inside the medium. It takes advantage of the ballistic propagation of ultrasound in biological tissues to access optical contrast with a millimeter resolution. However, acousto-optic signal is weak and presents a speckle pattern. Consequently, classical detection schemes based on interferometry with plane wave references lead to poor SNR. Thanks to photorefractive crystals, we developed a system based on wavefront adaptive holography that works around 800 nm and allows us to measure acousto-optic signal on a photodiode with a SNR 10 000 times higher. Due to its working at an appropriate wavelengths range inside the optical therapeutic window, such a technique is particularly well-suited for in-vivo applications. Among the medical fields we aim at exploring, one of the first we investigated is liver tumors imaging. Since their mechanical properties are very close to those of sound tissues around, it is difficult to see them with echography. Otherwise, invasive techniques must be used for optical imaging because of their location inside the human body. As a first step towards in-vivo imaging, we tested our setup on ex-vivo liver samples with tumors and obtained optical contrast as mechanical contrast was not significant.

8943-24, Session 4

Fiber-optic ultrasound transducers with carbon/PDMS composite coatings

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Miniature ultrasound imaging probes are widely used during minimally invasive procedures in the human body. They can provide high-resolution visualization of tissue microstructure to guide diagnosis or therapy in regions such as vessel lumens and heart chambers. Piezoelectric transducer elements are commonly used to generate and receive ultrasound, but their integration into medical devices with millimetre-scale dimensions can be challenging and expensive.

Recent studies have highlighted the potential for producing high ultrasound pressures by illuminating thin, light absorbing coatings using nanosecond-scale light pulses. This study is of the application of thin absorbing coatings to the distal ends of small diameter optical fibers which involves a unique set of challenges that is distinct from that encountered with larger surfaces. We have developed miniature transducers for transmitting and receiving ultrasound with pairs of optical fibres. The tip of the ultrasound generating fiber is coated with a composite of polydimethylsiloxane (PDMS) and carbon nanotubes or carbon black. The reception fiber uses an optical hydrophone comprising a Fabry-Perot cavity that is interrogated by a wavelength-tunable laser.

One- and two-dimensional measurements of biological tissue are presented, and we discuss the integration of these miniature, all-optical transducers into minimally invasive medical devices in combination with optical emission for photoacoustic sensing.

8943-25, Session 4

Multispectral photoacoustic imaging of nerves with a clinical ultrasound system

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Accurate and efficient identification of nerves is of great importance during many ultrasound-guided clinical procedures, including peripheral nerves blocks and prostate biopsies. Nerves are often poorly visible with conventional ultrasound imaging, however: they can be invisible or they have very similar appearances to surrounding structures such as tendons. Several recent studies have highlighted the potential for spectroscopic differentiation of nerves from surrounding tissues, based in part on the optical absorption of lipids that are present in intra- and extra-neural adipose tissue and in the myelin sheaths. These studies were limited to point measurements, however. In this study, a custom photoacoustic system with a clinical ultrasound imaging probe was used to acquire multi-spectral photoacoustic images of the nerves in the swine brachial plexus and surrounding tissues, across the wavelength range of 1100 to 1900 nm. Photoacoustic images were processed and overlaid in color onto co-registered conventional ultrasound images that were acquired with the same imaging probe. Pronounced optical absorption peaks centered at 1210 nm were observed in the photoacoustic signals obtained from nerves. These absorption peaks, which are consistent with the presence of lipids, provide a novel image contrast mechanism to significantly enhance the visualization of nerves. In particular, image contrast for nerves was over 5.5 times greater with photoacoustic imaging (0.82 ± 0.15) than with conventional ultrasound imaging (0.148 ± 0.002). This study demonstrates the potential of photoacoustic imaging to improve the clinical outcomes in ultrasound-guided interventions in regional anaesthesia and interventional oncology.

8943-26, Session 4

Correcting wavelength dependent optical fluence variations for deep tissue photoacoustic spectroscopy using reflection mode acousto-optics

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Photoacoustic spectroscopy (PAS) is capable of functional imaging in deep tissue and can distinguish locally between different intrinsic/extrinsic chromophores because of their distinct absorption spectra. A challenge is to make the technique quantitative, which cannot be achieved with PAS alone. This is because PA signals depend not only on local absorption coefficient but also on an unknown local fluence, which varies with the wavelength of the light used. This variation is a result of the wavelength dependence of the optical properties of the tissue. Our earlier work have shown that reflection mode acousto-optics is capable of measuring relative fluence and it can be combined with PA to compensate PA signals for fluence variations. We propose a method based on similar approach to compensate for wavelength dependent local fluence variations in PAS, which can further quantify the relative

concentration of chromophores, such as Oxygen saturation of blood and local uptake of contrast agents etc. Our method requires photoacoustic measurements to be done at multiple wavelengths which will probe the absorption nature of the chromophore involved followed by the reflection mode acousto-optics measurements at the same wavelengths to account for wavelength dependent fluence variations during PA measurements. The results of these PA and AO measurements normalized with input light power can then be used in one equations to extract quantitatively the saturation value of blood. We provide the proof of principle with phantom measurements, by measuring relative absorption coefficient of given chromophore at different wavelengths.

8943-27, Session 4

Temperature dependence of Gruneisen parameter in optically absorbing solutions measured by 2D optoacoustic imaging

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We propose a new methodology for accurate measurements of temperature dependence of Gruneisen parameter in optically absorbing solutions. The method is based on two-dimensional optoacoustic (OA) imaging, which allows improving accuracy of measurements and spatial localization of the studied samples as compared to a single-transducer sensing. We estimated OA response of optically absorbing solutions by measurements of median intensity on OA images within the region of interest as a function of temperature. We showed that when normalized to its value at a particular temperature OA image intensity becomes an accurate metric reflecting temperature changes of Gruneisen parameter regardless of local optical fluence and absorbance, assuming those remain constant with temperature. To validate the proposed methodology, we studied temperature dependence of diluted aqueous solutions of pentahydrate cupric sulfate in the range from 22 to 4oC. Our results perfectly matched known temperature dependence for the Gruneisen parameter of water. Using the developed methodology, we also found that Gruneisen-temperature relationship for cupric sulfate exhibit linear trend with respect to the concentration. We applied the same protocol to aqueous solutions of human hemoglobin irradiated with 800 nm laser, to assure invariance of the optical absorption with respect to temperature and oxygenation. As in the case of ionic solution of cupric sulfate, the hemoglobin results revealed linear relationship between normalized optoacoustic intensity and temperature in the range of 4 - 22oC. In addition to accurate measurements of Gruneisen changes with temperature, the developed technique provides a basis for future high-precision OA temperature mapping during hypo- and hyperthermia in live tissues.

8943-110, Session PSun

High-throughput fiber-array transvaginal photoacoustic probe for in vivo ovarian cancer imaging

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A high-throughput photoacoustic imaging probe for delivering high contrast and signal-to-noise ratio images is presented. The probe consists of a transvaginal ultrasound transducer integrated with a custom-made sheath. The sheath encases the transducer and is lined with highly reflecting (>85%) aluminum for high-intensity light output. The lower end of the sheath is tapered down to 10-degree for easy penetration into body cavities, and measuring 24mm across. Additionally, it has four, 1-mm core optical fibers surrounding it on the inside for tissue illumination. The probe design was optimized by simulating the emitted light fluence distribution for fibers having different numerical apertures and distances from the probe base using Zemax. This ensured high light intensity output with coupling efficiency of 83% and uniformity while at the same time remaining below the maximum permissible exposure recommended by ANSI. The performance of the probe was first evaluated by experimental measurements of the fluence with a CCD-camera. This was done through a 10mm chicken breast layer over intralipid solution for depths of up to 1cm. A 0.86mm-inner-diameter polyethylene tubing filled with blood, was successfully imaged to depths of up to 22mm in chicken breast using a fluence of 20 mJ/cm2 at 750nm. In-vivo mouse tumor model was performed and the photoacoustic image demonstrated regions of high and low oxygen saturation around the tumor. Lastly, ex-vivo images of a human ovary showed blood vasculature that correlated well with histology. These results suggest that our probe has great potential for in-vivo imaging and characterization of ovarian cancer.

8943-111, Session PSun

Optoacoustic monitoring of central and peripheral venous oxygenation during simulated hemorrhage

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Circulatory shock may be fatal unless promptly recognized and treated. The most commonly used indicators of shock (hypotension and tachycardia) lack sensitivity and specificity. In the initial stages of shock, the body compensates by reducing blood flow to the peripheral circulation e.g, skin and muscle in order to preserve vital organ perfusion e.g., brain and heart. Characteristically, this can be observed by a greater reduction in peripheral venous oxygenation (axillary vein) compared to central venous oxygenation (internal jugular vein). While invasive measurements of oxygenation are accurate, they lack practicality and are not without complications. We have developed a novel optoacoustic system that noninvasively determines oxygenation in specific veins. In order to test this application, we used lower body negative pressure (LBNP), which simulates hemorrhage by exerting a variable amount of suction on the lower body, thereby reducing the volume of blood available for central circulation. Restoration of normal blood flow occurs promptly upon cessation of LBNP. Using two optoacoustic probes, guided by ultrasound imaging, we simultaneously monitored oxygenation in the axillary and internal jugular veins (IJV). LBNP began at -20 mmHg, thereafter was reduced in a step-wise fashion (up to 30 min). The optoacoustic measurements of axillary oxygenation decreased, whereas IJV oxygenation remained constant. These results indicate that our optoacoustic system may provide safe and rapid measurement of peripheral and central venous oxygenation and diagnosis of shock with high specificity and sensitivity.

8943-112, Session PSun

Investigation of a method for laser-induced ultrasound tomography that eliminates the need for ray-tracing

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In this work, we investigate a novel reconstruction method for laser-induced ultrasound tomography (UST) breast imaging that circumvents limitations of existing methods that rely on ray-tracing. There is

currently great interest in developing hybrid imaging systems that combine optoacoustic tomography (OAT) and UST. There are two primary motivations for this: (1) the speed-of-sound (SOS) distribution reconstructed by UST can provide complementary diagnostic information; and (2) the reconstructed SOS distribution can be incorporated in the OAT reconstruction algorithm to improve OAT image quality. However, image reconstruction in UST remains challenging. The majority of existing approaches for UST breast imaging involve ray-tracing to establish the imaging operator. This process is cumbersome and can lead to severe inaccuracies in the reconstructed SOS images in the presence of multiple ray-paths and/or shadow zones.

To circumvent these problems, we implemented a partial differential equation-based Eulerian approach to UST that was proposed in the mathematics literature but never investigated for medical imaging applications. This method operates by directly inverting the Eikonal equation without ray-tracing. A numerical implementation of this method was developed and systematically compared to existing reconstruction methods for UST breast imaging. We demonstrated the ability of the new method to reconstruct accurate SOS maps from TOF data obtained by a 3D hybrid OAT/UST imager built by our team.

8943-113, Session PSun

Improvement of axial resolution in time-reversed ultrasonically encoded (TRUE) optical focusing by using two ultrasound transducers

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Focusing light inside highly scattering media beyond the ballistic regime is a challenging task in biomedical optical imaging, manipulation, and therapy. This challenge can be overcome by combining ultrasonic modulation and optical phase conjugation to time reverse the ultrasonically encoded diffuse light to the ultrasound focus inside a turbid medium. In this technique—called TRUE optical focusing, a photorefractive crystal or polymer is used as the phase conjugate mirror for optical time reversal. Accordingly, a relatively long ultrasound burst, whose duration matches the photorefractive response time of the photorefractive material, is used to encode the diffuse light. With this long ultrasound burst, the TRUE focusing resolution along the acoustic axis is poor. In this work, we used two transducers, emitting two intersecting ultrasound beams at different frequencies, to modulate the diffuse light within the intersection volume at the beat frequency. We show that the light encoded at the beat frequency can be time-reversed and converge to the intersection volume. Experimentally, TRUE focusing with an acoustic axial resolution of ~1.2 mm was demonstrated inside turbid media, agreeing with the theoretical estimation.

8943-114, Session PSun

High-speed time-reversed ultrasonically encoded (TRUE) optical focusing into scattering media at 793 nm

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Time-reversed ultrasonically encoded (TRUE) optical focusing focuses light into scattering media by phase-conjugating ultrasonically encoded diffuse light. In previous works, the speed of TRUE focusing was limited to no faster than 1 Hz by the response time of the photorefractive phase conjugation mirror or the speed of the digital camera as well as the rate of data streaming; photorefractive-crystal-based TRUE focusing was also limited to the visible spectral range. These time-consuming schemes prevent this technique from being applied in vivo, since living biological tissue has a speckle decorrelation time on the order of a millisecond. In this work, using a Te-doped Sn2P2S6 photorefractive crystal at the near-infrared wavelength (793 nm), we achieved TRUE focusing in 25 ms, which was 39 times faster than previously reported. To further demonstrate TRUE focusing, we imaged an absorbing target placed between two scattering layers with a total thickness of two transport mean free paths. The high speed and the fact that the wavelength used lies within the optical therapeutic window may ultimately enable a wide range of in-vivo applications, including deep tissue imaging, photodynamic therapy, and optical manipulation.

8943-115, Session PSun

Optical clearing enhanced photoacoustic microscopy

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Photoacoustic microscopy (PAM) is a recently developed imaging modality that can provide structural, molecular, functional, and metabolic information. Based on its focusing mechanism, PAM can be divided into two different implementations: acoustic-resolution PAM (AR-PAM) and optical-resolution PAM (OR-PAM). In OR-PAM, due to strong light scattering in tissue, both the spatial resolution and maximum penetration depth deteriorate sharply with depth. To reduce the scattering, tissue optical clearing (TOC) techniques have been widely used in many high-resolution optical imaging modalities. In this paper, we propose to use glycerol as an optical clearing agent in OR-PAM. In a phantom experiment, a U.S. penny covered by a piece of mouse skin was imaged. After optical clearing, the signal was at least 3 times stronger. In an in vivo mouse experiment, after optical clearing, the sensitivity increased by 4.4 times and the lateral resolution increased by 2.5 times. Our results show that the imaging performance of OR-PAM can be greatly enhanced by optical clearing both in vitro and in vivo.

8943-116, Session PSun

Optimization of all-optical ultrasound detector

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Photoacoustic microscopy (PAM) has been extensively studied in the field of functional biomedical imaging for its capability in noninvasive label-free imaging of biological samples. Traditional piezoelectric transducer has been widely used in PAM systems. The application of all-optical ultrasound detection technique, which measures ultrasonic pressure through variations in optical resonance, in PAM has gained more interests due to its miniaturization capability, high sensitivity, broadband response, and larger detection angle. A large detection angle, and thus a large field-of-view (FOV) in all-optical ultrasonic detector is especially important in microscopy systems using high magnification objective lens, which, however, has not been studied systematically. In this work, we proposed a theoretical method to study the FOV of an arbitrary optical detector. This method can be applied to detectors with different geometrical shapes and dimensions, and their FOVs with respect to the working distances and frequency bands can be acquired. Here, we studied the optical ring resonator structure.

8943-117, Session PSun

Improvement of signal detection selectivity and efficiency in two-photon absorption-induced photoacoustic microscopy

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To improve the penetration depth in photoacoustic microscopy while preserving high spatial resolution, we have proposed two-photon absorption-induced photoacoustic microscopy (TP-PAM). However, in tissue imaging, unwanted one-photon photoacoustic signals impair the image constructed from the two-photon photoacoustic signals, because the cross-section of two-photon absorption is smaller that of one-photon absorption. To overcome this drawback, it is important to enhance (or extract) only the photoacoustic signals induced by two-photon absorption.

In this study, to improve detection selectivity and efficiency of two-photon photoacoustic signals, we clarified the dependence of TP-PAM signal intensity and image quality on detection frequency range and excitation pulse duration in detail. In addition, we improved the excitation and detection configuration by using confocal setup between optical and acoustic foci. The comparison among photoacoustic signals generated by femtosecond, sub-nanosecond and nanosecond optical pulses enabled us to find that the sub-nanosecond pulses are suitable for TP-PAM. This seems to be because pulse duration is also important to generating photoacoustic signals, though two-photon absorption needs relatively high peak power. With the optimization of frequency filtering, pulse duration, and excitation and detection configuration, the spatial resolution (depth resolution: 10.2 ± 0.2 um, transverse resolution: 5.9 ± 0.4 um) and signal sensitivity (about 8 times higher than non-confocal configuration) are improved.

8943-118, Session PSun

Miniaturized photoacoustic endoscope with transparent optical micro-ring resonator ultrasonic sensor

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By providing an interior view of an organ cavity non-invasively or minimal-invasively, endoscopes grant physicians a great opportunity to look directly inside the body, playing an indispensable role in today's medical diagnosis process. Photoacoustic endoscope offers unique diagnostic capabilities including pigment mapping, tumor angiogenesis monitoring and blood oxygenation quantification. Currently, piezoelectric transducers are widely used in all the reported photoacoustic endoscopes; however, piezoelectric transducers are optically opaque and have only limited sensitivity and bandwidth. In contrast, optical micro-ring ultrasonic sensor fabricated on a transparent substrate has demonstrated its higher sensitivity and ultra-broad detecting bandwidth, which potentially offers a better solution for photoacoustic endoscope. Along with its high detection abilities, the fiber-based sensor also promises potential of miniaturizing, allowing implementation of fully optical photoacoustic endoscope probes with only a few millimeters in dimension. In our system, the irritation pulsed laser is guided through optical fiber to the probe head, where it is focused by a graded index (GRIN) lens and directed to the target. A motor rotates the probe head to produce a 360o circular scan that is perpendicular to the endoscope to acquire a photoacoustic image.

8943-119, Session PSun

Inertial cavitation in theranostic nanoemulsions with simultaneous pulsed laser and low frequency ultrasound excitation

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Ultrasound-induced inertial cavitation is a mechanical process used for site-localized therapies such as non-invasive surgery. Initiating cavitation in tissue is usually difficult, however, requiring very high intensity focused ultrasound (HIFU) which can also raise safety concerns. A new type of theranostic nanoemulsion has been developed as a combined ultrasound/photoacoustic agent for molecular imaging that potentially can also help optimize molecularly targeted therapies using HIFU. It includes a nanoscale emulsion core encapsulated with a layer of gold nanospheres at the water/ oil interface. Its optical absorption exhibits a spectrum broadened up to 1100nm, opening the possibility that 1064 nm light can excite cavitation nuclei. If optically-excited nuclei are produced at the same time that a low-frequency ultrasound wave is at peak negative pressure, then highly localized therapies based on acoustic cavitation may be enabled at very low ultrasound pressures. We have tested this concept using a low-cost, low energy, portable 1064 nm fiber laser in conjunction with a 1.24 MHz HIFU transducer for simultaneous laser/ultrasound excitation of nanoemulsions. A series of experiments was implemented to optimize the combined laser/HIFU impact. Active cavitation detection from backscattered signals indicated that inertial cavitation can be initiated at very low acoustic pressures (much less than 1 MPa) when laser excitation coincides with the rarefaction phase of the acoustic wave, and that no cavitation is produced when light is delivered during the compressive phase. Results on nanoemulsion disruption of clots in vitro also demonstrated a potential clinical application of the technology.

8943-120, Session PSun

Imaging the distribution of photoacoustic contrast agents in vivo: a spectral unmixing approach

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Multispectral photoacoustic (PA) imaging allows for the high-resolution visualization of optical absorbers in vivo and non-invasively, and the ability to distinguish multiple absorbers with differing optical properties in the same tissue. Here we describe the use of a commercially available multispectral PA imaging system (Vevo® LAZR, FUJIFILM VisualSonics, Inc., Toronto) to image and spectrally unmix signals from various absorbers in vivo.

Absorption curves for various agents can be used to unmix the spectral data sets into their individual components which can then be overlayed for visualization and quantification. Intra-muscular injections of methylene blue (Sigma) and IRDye 800CW carboxylate (LI-COR Biosciences) were performed in the hindlimb of a mouse where 2D images were acquired every 5nm from 680 to 970nm. In addition, the pharmacokinetics of an intravenously administered multi-modality agent (IRDye 800CW) in the kidney was analyzed. IRDye 800CW 2-DG or IRDye 800CW carboxylate was administered by tail vein at a dose of 0.4 mM (200ul). 2D and 3D photoacoustic imaging of the kidney was performed before, immediately,

6 hours and 24 hours after injection. Histology was performed to verify results obtained with photoacoustics.

In all in vivo experiments, the signal from the various dyes was distinguishable using spectral unmixing and verified by comparing the spectral curves from ROIs selected based on the unmixed data sets to the spectral curves generated by agents in a phantom. In addition, the feasibility of using spectral unmixing to distinguish and quantify an agent in a pharmacokinetic study was demonstrated.

8943-121, Session PSun

Photothermal bleaching in time-lapse photoacoustic microscopy

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Photobleaching, a common phenomenon in fluorescence microscopy, occurs when a fluorophore permanently loses its ability to fluoresce due to long exposure to intense excitation light. On the one hand, photobleaching undesirably limits the observation time for monitoring a dynamic process; on the other hand, photobleaching can be beneficially employed in molecular diffusion or motion studies via techniques such as fluorescence-recovery after photobleaching (FRAP) or fluorescence loss in photobleaching (FLIP). Properly exploiting photobleaching requires a thorough understanding of its dependence on the excitation light exposure.

However, photobleaching is not exclusively a feature of fluorescence microscopy: It also exists in other imaging modalities. One example is photoacoustic microscopy (PAM), in which the contrast agents can also be intentionally photo-destructed using strong excitation light, resulting in a gradual reduction of photoacoustic signals during repeated scans.

To begin to exploit the photothermal bleaching in PAM, here we studied its dependence on the laser pulse energy, the absorber's diameter, and the laser pulse duration while the laser focal diameter was held constant. GNPs were chosen as the targets because they were widely used as contrast agents in photoacoustic imaging applications. Our results revealed that, within the linear excitation range, photothermal bleaching behaved differently before and after the GNPs were raised to their melting point. Below this critical point, the photothermal bleaching rate had weak dependence on the laser pulse energy; while above the melting point, the photothermal bleaching rate increased rapidly. Based on this finding, we suggest a method to determine the optimal excitation laser pulse energy for time-lapse photoacoustic imaging.

8943-122, Session PSun

Cross-optical-beam nonlinear photoacoustic microscopy

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Although the lateral resolution of a conventional optical resolution photoacoustic microscope (OR-PAM) is optical diffraction-limited, the system relies on time-resolved acoustic detection to provide axial resolution, which is typically only tens of microns. To improve the axial resolution, techniques using high-frequency, broad-bandwidth ultrasound transducers have been reported, achieving an axial resolution of ~8 μm. Nevertheless, a high-frequency transducer has a short working distance (~1 mm), which hinders in vivo imaging. Moreover, high-frequency ultrasound waves experience severe attenuation in biological tissue, yielding a low signal-to-noise ratio.

To avoid these limitations, here we present nonlinear PAM with cross-optical-beam illumination, used to perform optical sectioning. An excitation laser was split into two beams and focused onto the sample through two low-NA objectives from below and sideways. The beams coincided both in space and time at the cross-beam focal volume. To acquire an A-line, the sample was scanned along the depth axis. At each voxel, the chromophores were excited by a variety of pulse energies and the corresponding PA signals were collected by an ultrasound transducer (38 MHz bandwidth). Due to optical saturation and nonlinear thermal expansion, the PA amplitude depends nonlinearly on the laser fluence. To form an image, the data was fitted to a polynomial model. The axial resolution associated with the cubic of laser fluence was measured as 8.7 μm, a fourfold improvement over the acoustically determined value. Since this improvement is purely due to optical sectioning, no restrictions were imposed on the PA detection. Compared to previous methods using high-frequency transducers, the cross-beam nonlinear PAM enables a centimeter-scale working distance, with a comparable axial resolution.

8943-123, Session PSun

Experimental study of ultrasound-modulated scattering light using different frequencies ultrasound probes

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The focused ultrasound plays a role in localization and modulating the scattering light in ultrasound-modulated optical tomography (UOT). Both the modulation efficiency of scattering light and the spatial resolution of UOT are determined by ultrasound. In this paper, we used focused ultrasound of various frequencies on the scattering medium respectively, and had the incident lights passing though the ultrasonic focal area to gain optical signals for ultrasonic modulation by photomultiplier tube. The effect of repetition frequency and pulse energy of impulse ultrasound on the ultrasonic modulation of scattering light is derived through experiment. Furthermore, we compare the imaging sensitivity in UOT with 1, 2.25, 5 and 10MHz center frequency of impulse ultrasound. Experimental results demonstrate that the greater pulse energy of ultrasound, the higher the modulation efficiency. And the different pulse repeating frequency of ultrasound has little effected the modulated scattering light. The sensitivity was higher with 1MHz ultrasound than 2.25, 5 and 10MHz ultrasound respectively.

8943-124, Session PSun

High axial resolution photoacoustic imaging enabled by 300 MHz ring resonator detector

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Photoacoustic microscopy (PAM) is an emerging 3D imaging modality in which the optical contrast is detected ultrasonically, providing rich structural and functional information. Spatial resolution is a key parameter in PAM. Sub-micron transverse resolution has been demonstrated in PAM, but axial resolution has been limited as it is mainly determined by the bandwidth of the acoustic detector. We have previously demonstrated an 8 μm axial resolution using a polymer microring detector. Later on, by applying deconvolution method, researchers were able to push the record up to 7.6μm with a commercial 125MHz ultrasound transducer. However, the deconvolution method is sensitive to noise, thus restricting its general applications. The resolution can be further reduced to 5.8μm by surrounding the imaging target with low-sound velocity silicone oil, but

this approach is not biologically compatible. In this work, we demonstrate a new record of 2.7μm axial resolution using the microring resonator as an ultrasound detector without any post signal processing or additional sample treatment. Using a thin metal film irradiated by a sub-2 ns laser pulse to generate a sufficiently short photoacoustic signal, the impulse response of the microring detector is measured to have a FWHM of 1.8ns, which translates to an axial resolution of 2.7μm by considering the acoustic velocity in biological tissues. This result can be attributed to broadband response of the microring detector, which is calibrated to have bandwidth of over 300 MHz at –3 dB, and result in more than threefold improvement of axial resolution compared with our previous results.

8943-125, Session PSun

X-ray acoustic computed tomography (XACT) and medical applications

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We propose X-ray acoustic computer tomography (XACT), a new imaging modality, which combines X-ray contrast and high ultrasonic resolution in a single modality. It is known that X-ray scatter in tissue is much lower than that of optical photons. Therefore, it can achieve super-depth (> 10 cm) high-resolution acoustic imaging when the x-ray energy exceeds 200 keV.

Imaging system was designed for XACT with very short pulsed x-ray beams (60 nanoseconds pulse width) at the energy of 270 kVp with a 15 Hz repetition rate. The X-ray acoustic signal generated by the X-ray pulses will be captured by unfocused ultrasound transducer. The transducer, driven by a computer-controlled step motor to scan around the sample, will detect the X-ray acoustic signals in the imaging plane at each scanning position. A pulse preamplifier will receive the signals from the transducer and deliver the amplified signals to a secondary amplifier. Signals will be recorded and averaged 128 times by oscilloscope. A sampling rate of 100 MHz was used to record 2500 data points at each view angle. All control codes will be written using Labview graphic programming language. One set of data incorporated 200 positions as the receiver moved 360°. The XACT image will then be reconstructed with the filtered back projection algorithm.

XACT has great potential to revolutionize the conventional X-ray imaging and provide a new tool for radiation dose monitoring in radiation therapy of cancer.

8943-126, Session PSun

Combined photoacoustic and speed-of-sound imaging using integrating optical detection

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Photoacoustic (PA) and ultrasound (US) imaging use the same instrumentation for the detection of acoustic waves and can therefore be combined easily. While PA images mainly reveal the optical absorption properties of an object the contrast in US images is given by its mechanical properties. In particular, speed-of-sound (SOS) maps can be obtained upon the detection of US pulses that have traversed the sample.

The laser ultrasound (LUS) method uses the PA effect for the generation of the incoming US waves at optically absorbing targets in front of the sample. For simultaneous SOS and PA imaging part of the same laser pulse that is used to illuminate the object is used for the illumination of these external absorbers.

In the presented setup a free laser beam, which is part of a Mach-Zehnder interferometer, is used for the detection of the US signals coming from and passing through the sample. Due to a special arrangement of flat or curved absorbing targets for LUS generation a single laser pulse yields information for a projection of the SOS distribution. The resolution is determined by the number of LUS sources. Separation of the LUS signals arriving at the integrating detector is possible because of their different times of flight. Reconstruction of a two-dimensional SOS distribution is then accomplished by applying an inverse Radon transform to the projections.

Different absorber geometries yielding plane and focused incoming LUS waves are tested. The knowledge of the SOS distribution can be used to improve the reconstruction of the PA image where typically a constant average value is assumed.

8943-127, Session PSun

PLGA/PFC particles loaded with gold nanoparticles as dual contrast agents for photoacoustic and ultrasound imaging

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Phase-change contrast agents consisting of a perfluorocarbon (PFC) liquid core stabilized by a lipid, protein, or polymer shell have been proposed for a variety of clinical applications. Previous work has demonstrated that droplet vaporization can be induced by laser irradiation through optical absorbers incorporated inside the droplet. In this study, Poly-lactide-co-glycolic acid (PLGA) particles loaded with PFC liquid and silica-coated gold nanoparticles (AuNPs) were developed and characterized using photoacoustic (PA) methods.[[ ]]

Microsized PLGA particles loaded with PFC liquid and AuNPs (14, 35, 55nm each with a 20nm silica shell) were synthesized using the double emulsion method. The PA signal intensity and optical vaporization threshold were investigated using a 375 MHz transducer and a focused 532- nm laser (up to 450-nJ per pulse). PLGA particles were then incubated with MDA cells for 2 hours to investigate passive targeting, and the vaporization threshold of the PLGA particles internalized within cells.[[ ]]

The laser-induced vaporization threshold energy decreased with increasing AuNP size. The vaporization threshold was 0.42, 0.69 and 0.85 nJ/cm2 for 5μm-sized PLGA particles loaded with 13.9±2.4, 34.9±2.5 and 54.5±4.2nm AuNPs, respectively. The PA signal intensity increased as the laser fluence increased. This trend was observed for all particles sizes. The PLGA particles passively internalized by MDA cells after 2-hour incubation time were visualized via confocal fluorescence imaging. Upon PLGA particle vaporization, bubbles formed inside the cells resulting in cell destruction. This work demonstrates that AuNP-loaded PLGA/PFC particles have potential as PA theranostic agents in PA imaging and optically-triggered drug delivery systems.

8943-128, Session PSun

Ultrasound modulated light blood flow measurement using intensity autocorrelation function: a Monte-Carlo simulation

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Development of techniques for continuous measurement of regional blood flow, and in particular cerebral blood flow (CBF), is essential for monitoring patients in critical care. Recently, a novel technique , based on ultrasound modulation of light was developed for non-invasive, continuous CBF monitoring (termed ultrasound-modulated light (UTL)), and shown to correlate with readings of laser Doppler. Coherent light is

introduced onto the tissue concurrently with an Ultrasound (US) field. Displacement of scattering centers within the sampled volume induced by Brownian motion, blood flow and the US field affects the photons' temporal correlation. Hence, the temporal fluctuations of the obtained speckle pattern provide dynamic information about the blood flow.

We developed a comprehensive simulation, combining the effects of Brownian motion, US and flow on the obtained speckle pattern. Photons trajectories within the tissue are generated using a Monte-Carlo based model. Then, the temporal changes in the optical path due to displacement of scattering centers are determined, and the corresponding interference pattern over time is derived. Finally, the light intensity autocorrelation function of a single speckle is calculated, from which the tissue decorrelation time is determined. The simulation's results are compared with in-vitro experiments, using a digital correlator, demonstrating decorrelation time prediction with errors smaller than 5% (in solutions with glycerol concentration of 80% and higher). This model may assist in the development of optical based methods for blood flow measurements and particularly, in methods using the acousto-optic effect.

8943-129, Session PSun

Dual-mode optical-resolution photoacoustic microscopy and photoacoustic tomography imaging system

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The simultaneous acquisition of photoacoustic images in both tomography mode (PAT) and optical resolution microscopy mode (OR-PAM) offers a number of advantages. For example, in skin imaging, combining both modes offers the prospect of achieving the large penetration depth of PAT required to visualize relatively deep large subcutaneous vessels whilst the micron scale lateral resolution provided by OR-PAM could reveal the dermal/epidermal microvasculature at capillary level. Achieving this type of dual mode imaging capability is challenging using conventional piezoelectric detectors due to their opacity. This makes it difficult to arrange the detectors in such a way that allows the tissue to be irradiated simultaneously with both the wide field beam required for PAT and the focused beam required for OR-PAM. A dual mode PAT/OR-PAM scanner based on a transparent Fabry-Perot (FP) ultrasound sensor has been designed that overcomes this limitation. A key feature is a novel dichroic coating design used to form the mirrors of the FP sensor. This extends the range of wavelengths at which the sensor is transparent to permit transmission at the optimal wavelength range (560-600nm) required for OR-PAM vascular imaging. It permits dual mode PAT/OR-PAM images to be obtained in vivo for the first time. The system provides a PAT FOV of 15x15mm with acoustically defined resolution in the range 50-150μm and 5x5mm OR-PAM FOV with an optically defined lateral resolution of 10μm. To demonstrate its potential application in dermatology, the scanner has been evaluated by imaging the mouse ear and acral skin in both modes.

8943-130, Session PSun

Photoacoustic saturation effect and the selection of ultrasonic detector

Hao F. Zhang, Hao Li, Biqin Dong, Zhen Zhang, Cheng Sun, Northwestern Univ. (United States)

Photoacoustic imaging is becoming increasingly important in quantitatively imaging in vivo functional tissue parameters, particularly the hemoglobin oxygen saturation (sO2). Current approaches in quantifying sO2 rely on the assumption of linear relationship between the optical energy deposition within a blood vessel and the measured photoacoustic signal amplitude. We investigated the conditions that an acoustic detector has to satisfy in order for a specific photoacoustic imaging system to accurately quantify sO2. In our study, we tested both commercial and custom-made piezoelectric ultrasonic detectors and further compared them with our transparent optical micro-ring resonator detector. We found that the transparent optical micro-ring detector offered the largest bandwidth and is, therefore, optimal for sO2 quantification. Moreover, the optically transparent nature of our micro-ring detector enabled it to be easily integrated with other optical microscopic modalities.

8943-131, Session PSun

Listen to photon propagation in biological tissues: quantitative optical scattering imaging and high-resolution diffuse optical tomography using photoacoustic measurements

Zhen Yuan, Univ. of Macau (Macao, China)

In this study we propose and experimentally validate a method for quantitative scattering coefficient imaging using photoacoustic data from one-wavelength illumination. The reconstruction method developed combines conventional PAT with the photon diffusion equation in a novel way to realize the recovery of scattering and absorption coefficients. We demonstrate the method using various objects having scattering contrast only or both absorption and scattering contrasts embedded in turbid media. The developed method described will be able to provide high resolution scattering imaging for various biomedical applications ranging from breast and joint to brain imaging. In particular, the proposed method is capable of recovering both absorption and scattering coefficients with zero crosstalk between these two different contrasts.

8943-132, Session PSun

Cross-correlation-based flowmetry using optical-resolution photoacoustic microscopy with a digital micromirror device

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Noninvasive and accurate blood flow measurement is critical for medical diagnosis. We proposed a cross-correlation-based method to quantitatively measure transverse flow velocity, using optical-resolution photoacoustic microscopy with a digital micromirror device (DMD). The DMD alternately delivers two spatially separated laser beams to the target. The slow-time photoacoustic signal profiles measured from the two beams are cross correlated. The magnitude and sign of the time shift in the cross-correlation profile are used to simultaneously calculate the speed and direction of transverse flow.

The proposed method was first demonstrated in an aqueous suspension of microspheres flowing in capillary tubing. Using 10-μm-microspheres, transverse flows in the range of 0.50–6.84 mm/s were measured with a root-mean-squared accuracy of 0.22 mm/s. Using three different sizes of microspheres (diameters: 3, 6, and 10 μm), we proved experimentally that the flow measurements were independent of the particle size for flows in the velocity range of 0.55–6.49 mm/s. We also observed an expected parabolic distribution of flow velocity along the depth direction. Both maximum and minimum measurable velocities were investigated for varied distances between the two beams and over varied total times for one measurement. In optically scattering chicken breast tissue, this technique showed an accuracy of 0.35 mm/s at 0.3 mm depth. Finally, we used this method to measure blood flow in a mouse ear in vivo.

8943-133, Session PSun

Photoacoustic microscopy for quantitative evaluation of angiogenesis inhibitor

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It is a well-known fact that medications are used to cure disorders. The investigations on quantitative evaluation of physiological changes through applying medications are essential to study the effectiveness of treatment. The disorders associated with neo-vasculature formation are important alterations in some diseases such as cancer, exudative age-related macular degeneration or rheumatoid arthritis. Because an increasing number of anti-angiogenic drugs are under development in order to treat diseases related to neoangiogenesis, e.g., cancers, the ability to quantify the result of angiostatic treatment plays a key role in assessing the effects of angiogenesis inhibitors. In this work, the feasibility of photoacoustic microscopy (PAM) for quantitative evaluation of angiogenesis inhibitor was investigated on a chick embryo model in vivo. Different concentrations of the angiogenesis inhibitor, Sunitinib, were applied to the chorioallantonic membrane (CAM) of the chick embryos. Imaging of microvasculature in embryo CAMs was acquired using a laser-scanning PAM system; while the optical microscopy (OM) served as a gold standard to capture microvascular images of the same set of CAMs for comparisons. The inclination of vessel density for different concentrations of Sunitinib obtained by PAM and OM are in good agreement, suggesting that PAM may provide an unbiased quantification of microvessel density. Moreover, we demonstrated the applicability of PAM in three-dimensional analysis of vessel density, which is highly restricted by OM due to superficial imaging depth. PAM for studying angiogenesis inhibitor may offer important information on the drug's efficacy in cancer treatment.

8943-134, Session PSun

Improving visibility in photoacoustic imaging with dynamic speckle illumination

Jérôme Gateau, Thomas Chaigne, Ori Katz, Sylvain Gigan, Emmanuel Bossy, Institut Langevin (France)

Photoacoustics provides images of photoabsorption, which are usually speckle-free for structures filled with densely packed absorbers (as blood vessels) and homogeneously illuminated. It is well known then that some anatomical structures may not be visible because of their large size or limited view issues. On the other hand, speckled images are for instance of primary importance for tissue characterization in ultrasound imaging. Generally in imaging, speckled images results from randomly distributed sub-resolution source (or scatterers). In the context of photoacoustic imaging, we propose to exploit a speckled illumination to enable the visualization of large homogenous structures or complex structures with multiple orientations, with a high-frequency and limited-view system. Optical speckle patterns were generated with a 532 nm nanosecond coherent laser passing through a rotating diffuser, and were used to illuminate the samples. Ultrasound detection of photoacoustic waves was done with a 20 MHz 128-element linear array. Two imaging phantoms, comprised respectively of a 5 mm diameter homogeneous absorbing inclusion and a 20 µm diameter thread arranged in a knot, were imaged for 50 realizations of the speckle illumination. By measuring the dispersion over the realizations of each pixel value derived from a photoacoustic reconstruction algorithm, we showed that the shape of both absorbing structures could be retrieved faithfully, with a speckle size down to 3 µm. The results were compared to those obtained with a homogeneous illumination. In conclusion, we showed that speckled illumination can compensate for imaging artifacts of photoacoustic imaging with incoherent illumination and reveal normally invisible features.

8943-135, Session PSun

Consecutive reconstruction strategy for estimating absorption and scattering coefficient distribution in multiple-illumination photoacoustic tomography (MI-PAT)

Peng Shao, Tyler J. Harrison, Roger J. Zemp, Univ. of Alberta (Canada)

Quantitative photoacoustic tomography (qPAT), which refers to quantification of the optical properties, has been proved challenging due to the nature of the imaging modality. The nonlinearity between the measured initial pressure distribution and the optical property maps makes the problem even more complex. Multiple-illumination photoacoustic tomography, namely MI-PAT, was proposed by our group to stabilize the qPAT. In this paper, we present an alternative algorithm for reconstruction of absorption (?_a) and scattering coefficient (?_s^') distribution with the MI-PAT configuration. With this method, the two optical properties are consecutively recovered based on data sensed with multiple optical illuminations of a single wavelength. With this strategy, ?_a is recovered with the so-called least-squares fixed-point iterative algorithm, and ?_s^' is estimated with a linearized algorithm. Simulation studies show that the two optical property maps in a known turbid biological object can be faithfully reconstructed with fast convergence. It is also demonstrated that this strategy outperforms conventional methods in terms robustness to noise, and ability to overcome the cross-talk between the recovered absorption and scattering coefficient map.

8943-136, Session PSun

Absolute photoacoustic thermography in deep tissue

Junjie Yao, Haixin Ke, Stephen Tai, Lihong V. Wang, Washington Univ. in St. Louis (United States)

In cancer therapy, heat is an effective tool. Applied in radiotherapy and chemotherapy, hyperthermia can increase the efficacy of drug delivery. Alternatively, thermotherapy can directly damage the tumor cells through thermoablation. In these thermal treatments, it is critical to monitor the deep tissue temperature in real time, so that the heating can be precisely controlled in order to reach the desired temperature and minimize damage to surrounding tissues. Photoacoustic (PA) thermography has been demonstrated as a promising tool for non-invasive temperature measurement in deep tissue, taking advantage of its high optical absorption contrast and low acoustic scattering. Existing PA temperature measurement, however, is limited to measuring relative temperature changes, because it is challenging to normalize the laser fluence in deep tissue. Here, we propose a new method on the basis of the dual temperature dependences of tissue Grüneisen coefficient and the speed of sound. While the former is reflected in the PA signal amplitude, the latter can be extracted from the PA signal duration. By taking ratiometric measurements at two adjacent temperatures, we can eliminate the factors that are temperature irrelevant but difficult to correct for, including the local laser fluence, absorption coefficient and target size. Thereby, the absolute temperatures can be estimated. To validate our method, absolute temperatures of a blood tube embedded in chicken tissue were measured with the temperature varied from 30 to 45 °C.

8943-137, Session PSun

Reconstruction of the optical absorption coefficient from photoacoustic signals measured by scanning coaxial probe with regularization methods

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Reconstruction of the distribution of the absorption coefficient from photoacoustic (PA) signals is discussed. The photoacoustic signals were acquired by using a ring-shaped acoustic sensor made of P(VDF-TrFE) film. An optical fiber was coaxially arranged with the acoustic sensor. A nano-second pulsed laser illuminated the imaging object via the optical fiber. The acoustic sensor with the optical fiber scanned the measured object and obtained the PA signals at multiple positions for the successive image reconstruction. The distribution of the absorption coefficient was reconstructed by solving an inverse problem based on the PA wave equation. The linear forward model related the PA signal source to the PA signals for transparent medium such as water was formulated by solving the PA wave equation with finite element method. For the imaging object with scattering medium such as biological media and phantom made of water and intralipid, the light propagation should be considered to reconstruct the absorption coefficient. The light propagation was described with the photon diffusion equation (PDE). The relation of the absorption coefficient and the PA signal sources was linearized based on PDE. Then the linearized forward model combining PA wave equation and PDE was formulated. The distribution of the absorption coefficient was reconstructed by solving the inverse problems. The inverse problem was solved with some regularization methods such as the Tikhonov regularization and the lp sparsity regularization. The reconstructed images with the regularization methods were compared from the standpoints of spatial resolution, robustness to noise and quantification of the absorption coefficient.

8943-138, Session PSun

Image reconstruction of photoacoustic tomography based on finite-aperture-effect corrected compressed sensing algorithm

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In this study, we proposed a new compressed sensing (CS) based image reconstruction method for photo-acoustic tomography (PAT). To eliminate the finite aperture effect, the proposed method adopts the spatial impulse responses (SIRs) of the finite-sized flat transducer into the linear discrete PAT imaging model for CS. By using the nonlinear recovery algorithm based on convex optimization, PAT can be reconstructed with highly incomplete data. Therefore, the number of measurements and the system cost needed for a given image quality can be significantly reduced. In the mean time, retrospective restoration of the tangential resolution can be achieved because the SIR effect is incorporated in the CS. Simulation results demonstrate that this method not only reduce the data acquisition time but also improve the degraded tangential resolution for PAT with finite-sized flat transducers.

8943-139, Session PSun

Cross-sectional optoacoustic tomographic reconstructions in a polar grid

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Accurate optoacoustic (photoacoustic) reconstruction is an essential issue to advance on the quantitative capacities of optoacoustic tomographic imaging systems. One of the most commonly employed geometrical configurations makes use of an array of cylindrically-focused transducers located around the imaging sample to selectively acquire the optoacoustic signals generated in the imaging plane. Thereby, simultaneous acquisition of signals leads to important advantages such as high-throughput performance or real-time imaging capacity. For this acquisition geometry, model-based reconstruction procedures have showcased improved performance over commonly employed back-projection formulae in terms of imaging accuracy and flexibility to model transducer effects and acoustic propagation phenomena. The forward model is expressed as a linear operator (model-matrix) that maps the optical absorption in a grid containing the sample to the resulting wavefield at the sensor positions, resulting generally in large memory requirements and long computation times. Herein, an optoacoustic model based on a discretization of the time-domain equation in a polar grid is introduced. Due to the rotational symmetries of the acquisition geometry and the discretization grid, only the part of the model-matrix corresponding to a transducer position (projection) needs to be stored. Then, inversion of the model-matrix can be done in a fast and memory efficient manner. Performance of the method was tested in numerical simulations and experimental measurements, resulting in equivalent results as when using a Cartesian grid but in a much more computationally efficient implementation.

8943-140, Session PSun

Longitudinal in vivo photoacoustic imaging of tissue regeneration using an all-optical scanner

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Preclinical biomedical imaging plays a key role in furthering our understanding of the biological processes involved in tissue regeneration and aids the development of new therapies for the functional recovery of injured organs. It has been shown that angiogenesis can be vital to the successful completion of the healing process. It is therefore advantageous to be able to visualize and to monitor its progression in small animal models in vivo in a longitudinal and noninvasive manner. However, imaging of angiogenesis without the use of contrast agents holds challenges for many of the current modalities, while histology-based investigations typically prevent longitudinal studies on the same animal. The absorption-based contrast of photoacoustic imaging has been shown to be highly suited to visualizing blood vessel growth. In this study, an all-optical photoacoustic scanner based on a Fabry-Perot polymer film ultrasound sensor was used to image angiogenesis in small animal models of musculoskeletal injury, such as muscle trauma and bone fracture. High resolution (tens of microns) 3-D tomographic images of the vascular structure in the rat leg were obtained in backward mode using near-infrared excitation wavelengths (600nm to 1000nm). Images were acquired over a period of a few weeks and show the vasculature to depths of several millimeters. Future applications of photoacoustic modalities in this field may be extended to imaging mesenchymal stem cells labelled using genetically expressed absorbing proteins and may therefore allow a correlation between stem cell activity and angiogenesis.

8943-141, Session PSun

Mouse brain structure imaging using photoacoustic computed tomography

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Photoacoustic computed tomography (PACT) has been used in small animal brain imaging for its ability to provide both structural and functional information. Even though PACT's relatively deep penetration depth and high resolution are its advantages over other optical imaging modalities, acoustic distortion induced by the skull, by the nasal cavity and by the ear canals largely limit the image quality in the deep brain. In our work, we presents ex vivo PACT images of freshly excised mouse brain. The purpose is to serve as a good reference for future in vivo studies. At a depth of 3 mm, brain structures such as striatum, hippocampus and inferior colliculus can be clearly differentiated. At a depth of 6 mm, an arterial structure called Circle of Willis, which is located at the bottom of the brain, can be seen. Our results show that if acoustic distortion can be accurately accounted for, PACT should be able to image the entire mouse brain with rich structural information.

8943-142, Session PSun

In vivo spectroscopic photoacoustic tomography imaging of a far red fluorescent protein expressed in the exocrine pancreas of adult zebrafish

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Fluorescent proteins brought a revolution in life sciences and biological research in that they make a powerful tool for researchers to study not only the structural and morphological information, but also dynamic and functional information in living cells and organisms. While green fluorescent proteins (GFP) have become a common labeling tool, red-shifted or even near infrared fluorescent proteins are becoming the research focus due to the fact that longer excitation wavelengths are more suitable for deep tissue imaging. In this study, E2-Crimson, a far red fluorescent protein whose excitation wavelength is 611 nm, was genetically expressed in the exocrine pancreas of adult zebrafish. Using spectroscopic all optical detection photoacoustic tomography, we mapped the distribution of E2-Crimson in 3D after imaging the transgenic zebrafish in vivo using two different wavelengths. With complementary morphological information provided by imaging the same fish using a spectral domain optical coherence tomography system, the E2-Crimson distribution acquired from spectroscopic photoacoustic tomography was confirmed in 2D by epifluorescence microscopy and in 3D by histology. To the authors' knowledge, this is the first time a far red fluorescent protein is imaged in vivo by spectroscopic photoacoustic tomography. Due to the regeneration feature of zebrafish pancreas, this work preludes the longitudinal studies of animal models of diseases such as pancreatitis by spectroscopic photoacoustic tomography. Since the effective penetration depth of photoacoustic tomography is beyond the transport mean free path length, other E2-Crimson labeled inner organs will also be able to be studied dynamically using spectroscopic photoacoustic tomography.

8943-145, Session PSun

Transparent broadband ultrasonic detector for functional photoacoustic imaging

Hao Li, Biqin Dong, Zhen Zhang, Siyu Chen, Cheng Sun, Hao F. Zhang, Northwestern Univ. (United States)

Functional photoacoustic microscopy (PAM) has been extensively studied for its capability in noninvasive label-free imaging of biological samples in three-dimension (3D) due to its physiologically relevant optical-absorption contrast and high spatial resolutions. The rapid advance motivates the integration of PAM with existing optical imaging modalities for more comprehensive clinical diagnosis and biomedical research, which further requires high performance ultrasound detectors without obstructing the optical path. However, traditional piezoelectric detectors, which are opaque and, sometimes, bulky, are incompatible with constrained physical dimensions in optical microscopic systems. Although it is possible to create opening at the center of a detector to reduce obstruction to the imaging path, their bandwidths and angular sensitivities are still limited. In contrast, using light to demodulate photoacoustic signal provides a better solution, where optical detector can offer broader detection bandwidth in a miniaturized and optically-transparent form. In this paper, we present a systematic study of an optically-transparent ultrasound detector using a polymeric micro-ring resonator on a 250-?m thick microscope coverslip. Our analysis formulates the general guideline for the design of optical transducer. The optimal design was further validated experimentally for its key sensing characteristics including sensitivity, bandwidth, angular dependence, and functional imaging capabilities including lateral/axial resolution and saturation limit. Such a transparent ultrasonic detector opens a new window for developing multi-modal optic/photoacoustic imaging systems for both clinical applications and biological researches.

8943-146, Session PSun

Magnetically mediated thermo-acoustic imaging

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Thermoacoustic imaging has undergone galvanizing progress in recent decades without sign of slowing down. Different spectrum of electromagnetic waves, including light, microwaves, have been used for inducing thermoacoustic effect for imaging and, due to the distinct interactions between these electromagnetic waves with human tissues, different contrasts for diagnosis are extracted. In this paper, alternating magnetic field is explored for inducing thermoacoustic effect on conductive objects. Termed as magnetically mediated thermo-acoustic (MMTA) effect that provides a contrast in conductivity, this approach employs magnetic resonance for delivering energy to a desired location by applying a large transient current at radio frequency below 50MHz to a compact magnetically resonant coil. The alternating magnetic field induces large electric field inside conductive objects, which then undergoes joule heating and emanates acoustic signal thermo-elastically. The magnetic mediation approach with low radio frequency can potentially provide deeper penetration than microwave radiation due to the non-magnetic nature of human body and therefore extend thermoacoustic imaging to deep laid organs. Both incoherent time domain method that applies a pulsed radio frequency current and coherent frequency domain approach that employs a linear chirp signal to modulate the envelop of the current are discussed. Owing to the coherent processing nature, the latter approach is demonstrated capable of achieving much better signal to noise ratio and therefore potential for portable imaging system. The theory behind MMTA is described and phantom experiments are carried out to demonstrate the signal generation together with some preliminary imaging results. Ex-vivo tissue studies will also be investigated.

8943-147, Session PSun

All-optical intravascular optoacoustic catheter

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A coronary-artery compatible optoacoustic catheter is demonstrated. The catheter is composed of two optical fibers: one to provide optical excitation and the other to detect the resulting ultrasound using interferometry. The detection element is pi-phase-shifted fiber Bragg grating with an effective length of 350 µm written in a single-mode fiber. Pulse interferometry in reflection mode is used for the interrogation of the ultrasound sensor. Illumination is provided by a multi-mode fiber with a prism mounted on its tip for side illumination. Both fibers are housed in a dual-lumen tube with a diameter of approximately 1 mm and aligned to achieve maximum overlap between the illumination and the detection field of the ultrasound sensor. Since the ultrasound sensor has radial symmetry and therefore cannot discriminate between signals originating at different angles, imaging is performed by rotating only the illumination fiber. Thus, angular resolution in this configuration is determined solely by the optical beam divergence, which in our design is approximately 10°. Excitation is performed at an average power of 15 mW with pulses at a rate of 1.4 kHz, enabling imaging at a rate of 200 revolutions per minute. The performance of the catheter is demonstrated in imaging a stented blood vessel ex vivo. Despite vibrations in the catheter caused by the rotation of the illumination fiber, stable operation was maintained during the imaging session.

8943-148, Session PSun

Multimodal non-contact photoacoustic and OCT imaging using a fiber based approach

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Optical Coherence Tomography (OCT) is a high-resolution and contactless imaging method. It allows the acquisition of depth resolved images of (sub)surface features in turbid media. OCT employs the partial coherence properties of a broadband light source and interferometry to detect reflected light from backscattering interfaces. In photoacoustic imaging (PAI) ultrasonic waves, generated by rapid thermoelastic expansion due to absorption of short laser pulses, are usually acquired using ultrasonic transducers. These transducers require acoustic coupling with the specimen usually provided by water or other coupling agents. A combination of OCT and PAI ideally should not rely on contacting means to make full use of the non-contact nature of OCT. As alternative to contacting transducers remote (or non-contact) photoacoustic imaging techniques can be used which allow measurement of ultrasonic displacements on a sample surface without the need for a coupling agent.

In this work we acquire photoacoustic signals remotely on the surface of a specimen by an interferometric technique. The interferometer is realized in a fiber-optic network using a laser with a wavelength of 1550nm. In the same fiber-optic network a Fourier-domain OCT (FD-OCT) system is realized. The FD-OCT system employs a superluminescent diode with a wavelength of 1310nm as light source; the reflected light is acquired using a spectrometer with an InGaAs line array. Multimodal photoacoustic and OCT imaging is demonstrated on tissue mimicking phantoms and biological samples. As the same fiber network and optical components are used for photoacoustic and OCT imaging the obtained images are perfectly co-registered.

8943-227, Session PSun

Increase of penetration depth in real-time clinical epi-optoacoustic imaging: clutter reduction and aberration correction

Michael Jaeger, Sara Peeters, Gerrit Held, Michael Gruenig, Martin Frenz, Univ. Bern (Switzerland)

In a multi-modal combination with pulse-echo ultrasound (US), optoacoustic (OA) imaging is promising to improve diagnostic accuracy via the display of small blood vessels and local blood oxygenation within the anatomical context shown on US. Epi-mode OA, where the irradiation optics is integrated into the acoustic probe, allows most flexible clinical application on any part of the body that is already accessible to US. Unfortunately, signal clutter originating from the site of tissue irradiation deteriorates epi-OA image contrast, often limiting the penetration depth to around one centimeter. We have implemented displacement-compensated averaging (DCA) for clutter reduction on a research ultrasound system, and we demonstrate that DCA significantly improves image contrast as compared to conventional averaging in real time scanning of human volunteers with freehand probe guidance. This demonstrates that clutter reduction becomes a basic requirement for successful deep clinical epi-OA imaging. Acoustic aberrations are a further cause of low contrast, caused by an inhomogeneous speed of sound in the tissue. We will present a novel method that allows determining the distribution of speed of sound spatially resolved and in real-time using pulse-echo US. Based on this information efficient aberration correction and thus further improved resolution and contrast is possible in deep epi-OA imaging.

8943-228, Session PSun

The origin of clutter in in-vivo epi-optoacoustic imaging

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In a multi-modal optoacoustic (OA) and pulse-echo ultrasound (US) imaging system, epi-mode irradiation with the irradiation optics integrated into the acoustic probe has the advantage of clinically being applicable in a flexible way on any part of the body that is accessible to US. We have previously shown in realistic phantom experiments that the epi-OA images contain significant clutter signals, which strongly deteriorate image contrast. In an explorative study using a real-time combined OA and US system, we have now thoroughly investigated clutter in automated scanning of the forearm of human volunteers. The results demonstrate that clutter is also significant in clinical scanning. Different origins of clutter have been elaborated: OA transients that originate in the site of tissue irradiation and are detected by the transducer directly (direct clutter) as well as via acoustic scattering when propagating into the tissue (echo clutter). We also show that the signal-to-clutter ratio strongly depends on the separation distance between the imaging plane and the irradiation region, resulting in an optimum at an intermediated irradiation distance.

8943-28, Session 5

Three-dimensional hand-held optoacoustic imaging of spectrally-distinctive human structures in real-time

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We report on a novel hand-held imaging probe for real-time optoacoustic

visualization of deep tissues in three dimensions. The proposed solution incorporates a two-dimensional array of ultrasonic sensors densely distributed on a spherical surface, whereas illumination is performed coaxially through a cylindrical cavity in the array. Visualization of three-dimensional tomographic data at a frame rate of 10 images per second is enabled by parallel recording of 256 time-resolved signals for each individual laser pulse along with a highly efficient GPU-based real-time reconstruction. A liquid coupling medium (water), enclosed in a transparent membrane, is used to guarantee transmission of the optoacoustically generated waves to the ultrasonic detectors. Excitation at multiple wavelengths further allows imaging spectrally distinctive tissue chromophores such as melanin, oxygenated and deoxygenated haemoglobin. The performance is showcased by video-rate tracking of deep tissue vasculature, real-time imaging of hemodynamic changes and three-dimensional measurements of blood oxygen saturation in a healthy human volunteer. The flexibility provided by the hand-held hardware design, combined with the real-time operation, makes the developed platform highly usable for both small animal research and clinical imaging in multiple indications, including cancer, inflammation, skin and cardiovascular diseases, diagnostics of lymphatic system and breast.

8943-29, Session 5

Photoacoustic imaging of the carotid artery plaque

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The human carotid artery (CA) is one of the main arteries of interest to cardiovascular research. The biological constituents that make up the artery wall are important parameters for the clinical evaluation of the carotid artery. Classification of an arterial plaque to be either stable or unstable depends heavily on the tissue composition. Photoacoustic (PA) imaging could potentially be used to image the relevant biological plaque constituents [1,2]. In this study we evaluate experimentally the possibility of PA imaging of the carotid artery. We propose to image the carotid artery by means of internal illumination (via the throat) and external (neck side) ultrasound detection. For this purpose we built a PA carotid imaging system comprising a small 1.5 mm side-firing optical probe and a conventional linear ultrasound array (Vermon) interfaced with an open ultrasound system (Lecoeur Electronique). We experimentally tested this system by imaging five different carotid artery plaques obtained from carotid endarterectomy. Spectroscopic PA imaging was performed on all plaques revealing plaque constituents such as lipids and thrombus. To analyse the time-pressure profiles resulting from the plaques experiments another set of experiments was performed with a calibrated hydrophone (Precision Acoustics). The results provide proof that PA imaging can be used to image the relevant plaque constituents. The imaging setup is ready for imaging the carotid artery in-vivo. These experiments are planned for the coming months.

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8943-30, Session 5

Detection of breast lesions using spectroscopic photoacoustic imaging in a transgenic mouse model

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An estimated 232,340 new cases of breast cancer, the second deadliest in women, will be diagnosed in 2013 in the USA. Although B-mode ultrasound (US) increases the detection rates, the positive predictive value is low. Photoacoustic imaging can add additional molecular information by imaging tissue chromophores. The potential of spectroscopic photoacoustic (PA) imaging for quantification of tissue oxygen saturation (sO2) in order to differentiate among varying breast histologies in a transgenic mouse model of breast cancer was explored in this study. The transgenic mouse model for breast cancer development FVB/N Tg(MMTV/PyMT634Mul was used to assess sO2 levels in four different breast histologies (normal, hyperplasia, ductal carcinoma in situ (DCIS), and invasive carcinoma) using spectroscopic photoacoustic imaging. Mammary glands (n=233) of female mice at varying ages (4-10 weeks), were imaged using the VisualSonics Vevo LAZR, collecting combined ultrasound and photoacoustic data (10 mJ/cm2 fluence, 700-860 nm, 10 nm increments) using a 25 MHz transducer. Using in-house data analysis, the average sO2 of each mammary gland was calculated. Glands were then excised, stained with H&E, and classified. Hyperplasia 49.8% ± 1.5%; p<0.0001), DCIS (42.9% ± 1.0%; p<0.0001), and invasive (44.1% ± 1.1%; p <0.0001) lesions showed a statistically significant increase of average sO2 over normal mammary tissues (34.8% ± 0.5%). These results show spectroscopic PA imaging in a transgenic mouse model of breast cancer development is feasible. The increased tissues oxygenation in varying histologies of breast tissues characterized with PA and US imaging may help in breast focal lesion characterization.

8943-31, Session 5

Clinical application of photoacoustic flow cytometer for detection of circulating melanoma cells in vivo and ex vivo

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The examination of a total blood volume in vivo could provide potentially detection one CTC in a whole human blood volume. This could reach an ultimate CTC detection limit of 1 CTC in 5 liter of blood that is approximately three orders of magnitude better than the sensitivity threshold of existing CTC assays. We developed the clinical prototype of in vivo photoacoustic (PA) flow cytometer (PAFC), which provides the assessment of 1-3 liter of blood during 45-60 minutes. The principle of PAFC is based on the irradiation of a hand blood vessel with near-infrared high pulse repetition laser and detection of laser-induced acoustic waves from single CTC with ultrasound transducer attached to skin. In this report we present first clinical results with focus on detection of CTCs in melanoma patients. The advantages of in vivo PAFC were validated through comparison with the results of existing ex vivo/in vitro tests including in vitro PAFC and immunohistochemical staining with antibody cocktails. In vivo PAFC method revealed a larger (3-5-fold) number of CTC-associated PA signals in whole blood, compared to other assays. This suggests that new approach is more accurate and rapid (≤ 1 hour) that conventional time-consuming (many hours) CTC assays requiring multiple processing and measuring steps (e.g., enrichment, separation, labeling, or washings) that result in substantial loss of CTCs. In addition, we present also application of described PA clinical prototype for in vivo dynamic monitoring of circulating iting clots.

8943-32, Session 5

Optoacoustic measurement of central venous oxygenation for assessment of circulatory shock: clinical study in cardiac surgery patients

Irene Y. Petrov, Donald S. Prough M.D., Michael Kinsky, Yuriy Y. Petrov, Andrey Petrov, S. Nan Henkel, Roger Seeton, Rinat O. Esenaliev, The Univ. of Texas Medical Branch (United States)

Circulatory shock is a dangerous medical condition, in which blood flow cannot provide the necessary amount of oxygen to organs and tissues. Currently, its diagnosis and therapy decisions are based on hemodynamic parameters (heart rate, blood pressure, blood gases) and mental status of a patient, which all have low specificity. Measurement of mixed or central venous blood oxygenation via catheters is more reliable, but highly invasive and associated with complications. Our previous studies in healthy volunteers demonstrated that optoacoustic systems provide non-invasive measurement of blood oxygenation in specific vessels, including central veins. Here we report the results of a clinical study in coronary artery bypass graft (CABG) surgery patients. We used our custom-made medical-grade OPO-based optoacoustic system to measure in real time blood oxygenation in the internal jugular vein (IJV) of these patients. A clinical ultrasound imaging system (GE Vivid e) was used for IJV localization. Catheters were placed in the IJV as part of routine care and blood samples taken via the catheters were processed with a CO-Oximeter. The optoacoustic oxygenation data were compared to the CO-Oximeter readings. Good correlation between the noninvasive and invasive measurements was obtained. The results of these studies suggest that the optoacoustic system can provide accurate, noninvasive measurements of central venous oxygenation that can be used for patients with circulatory shock.

8943-33, Session 5

Optoacoustic technique for noninvasive diagnosis of hematomas and monitoring cerebral venous blood oxygenation in patients with traumatic brain injury

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Evacuation of acute intracranial hematomas within four hours is recommended to increase the likelihood of an acceptable neurologic outcome in patients with traumatic brain injury (TBI). CT and MRI provide noninvasive diagnosis of intracranial hematomas, but cannot be used until a patient arrives at a major healthcare facility. More rapid, accurate diagnosis could substantially improve outcome. NIRS suggests the presence of unilateral intracranial hematomas, but provides minimal localizing information due to limitations associated with strong light scattering. We proposed to use optoacoustics for hematoma diagnosis. The optoacoustic technique combines high contrast with ultrasound resolution and may provide valuable information on hematoma location, size, and oxygenation. We built medical grade, multi-wavelength, OPO-based optoacoustic systems tunable in the 700-1064 nm spectral range and unique, highly-sensitive, wide-band (25 kHz – 10 MHz) probes for detecting optoacoustic waves induced in hematomas and the superior sagittal sinus (SSS). The system was tested in TBI patients. Optoacoustic signals from hematomas were detected in TBI patients and used for measurements of hematoma blood oxygenation. SSS blood oxygenation was measured as well. The obtained data suggest that the optoacoustic system can be used for early detection and characterization of hematomas in TBI patients and measurement of cerebral venous blood oxygenation. Moreover, these results indicate that the optoacoustic technique may be used for diagnosis of hemorrhagic and ischemic stroke.

8943-34, Session 5

Clinically translatable integrated ultrasound and photoacoustic imaging system

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Due to the high scattering coefficient of tissue over the wavelength range used for photoacoustic (PA) imaging, most studies employ bulky, low repetition-rate lasers to provide sufficient pulse energies at depth within the body. The size and cost of these lasers has impeded integration of photoacoustics into conventional, routinely used ultrasound (US) scanners.

Here, we present an approach leveraging the capabilities of modern, high repetition-rate fiber lasers to produce a clinically translatable system providing integrated PA/US images at frame rates > 30 Hz. The system uses a portable, low-cost, low pulse-energy (1 mJ/pulse), high repetition-rate (1 kHz), 1064 nm laser and is designed for integrated PA/US imaging of the peripheral vasculature. Using a rotating galvo-mirror system, the incident laser beam is quickly scanned over the imaging area. Multiple PA images covering the scan area are integrated through a weighting algorithm to form a single PA image. Additionally, ultrasound firings are interleaved within the scan sequence to provide an US image reconstructed over the same frame period.

We acquired PA images of a 2-mm diameter ink-filled (absorption coefficient 5 cm^-1) tube embedded in chicken breast tissue at 1-cm depth. A 2cm x 1cm (depth x width) image was reconstructed. We obtained a signal-to-noise ratio of 15 dB, comparable to conventional PA methods using high-energy, low repetition-rate lasers. The current system produces an integrated PA/US frame at a 32 Hz frame rate, and 100 Hz frame rates are possible with our present approach for peripheral vascular applications.

8943-35, Session 6

Optical resolution photoacoustic microscopy using capacitive micromachined ultrasonic transducer

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We present optical resolution photoacoustic microscopy using a single element Capacitive Micromachined Ultrasonic Transducer (CMUT). A multi-wavelength source consists of a diode-pumped, pulsed Ytterbium fiber laser and 3m single mode fiber is used. Taking advantage of stimulated Raman scattering, four different peaks at 532nm, 545nm, 560nm and 590nm with pulse repetition rates of up to 200kHz were generated. CMUTs are promising as next generation ultrasound transducers. They offer many potential advantages over piezoelectric transducer technology including high sensitivity (~mPa/√Hz), small element footprint, acoustic impedance matching, broad bandwidth, minimal heat build-up and ease of mass fabrication and integration with electronics. In this experiment, a single element sacrificial-release based CMUT with 5MHz center frequency and <100Pa sensitivity was employed. Carbon fiber networks with ~7.5µm diameter were imaged in a transmission mode. The resolution study showed ~ 7 ?m resolution. To the best of knowledge, this is the first time that OR-PAM images using

CMUTs are reported. The advantages of proposed system may be open up a new range of application for OR-PAM imaging. The small footprint of transducer and probe are sufficiently small to be incorporated in the accessory port of clinical endoscopes leading to new opportunities for clinical imaging of the microvasculature. In vivo studies are planned in the near future.

8943-36, Session 6

DMD-based random-access optical-resolution photoacoustic microscopy

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The scanning mechanism has always been a major technical focus in optical-resolution photoacoustic microscopy. Flexible scanning access with fast scanning speed is desired to monitor biological and physiological dynamics with high temporal resolution. We developed random-access optical-resolution photoacoustic microscopy (RA-OR-PAM) using a digital micromirror device (DMD). Each micromirror on the DMD can be independently controlled, allowing to image regions of interest (ROIs) with arbitrary user-selected shapes without extraneous information. A global structural image is first acquired, and the ROIs are selected. The laser beam scans these regions exclusively, resulting in a faster frame rate than in a conventional raster scan.

This system can rapidly scan arbitrarily shaped ROIs with a lateral resolution of 3.6 μm within a 40?40 μm2 imaging area, a size comparable to the focal spot size of a 50 MHz ultrasound transducer. We demonstrated the random-access ability of RA-OR-PAM by imaging two static samples, a carbon fiber cross and a monolayer of red blood cells, with an acquisition rate up to 4 kHz. This system was then used for structural and functional imaging of dynamic physiological processes. For structural imaging, we monitored blood flow in vivo in real time within user-selected capillaries in a mouse ear. By imaging only the capillary of interest, the frame rate was increased by up to 9.2 times. For functional imaging, we used this system to quantitatively measure the transverse blood flow in vivo.

8943-37, Session 6

Ultrasound-assisted thermal clearance photoacoustic flowmetry

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Recently, photoacoustic sensing and imaging of blood flow have attracted growing interest. Compared with other techniques, photoacoustic flowmetry provides high sensitivity to optical absorption and is easy to integrate with anatomical and functional photoacoustic imaging. Although optical-resolution photoacoustic flowmetry has been demonstrated in superficial biological tissue, it remains a challenge in deep tissue, where photoacoustic imaging modalities provide only acoustic spatial resolutions.

Here we present a new flow measurement method based on ultrasound heating and photoacoustic detection of the thermal clearance rate. An ultrasound transducer heats up a volume of the flow medium, and temperature-dependent photoacoustic signals from the heated volume are measured by an acoustic-resolution photoacoustic microscope. The flow speed is determined from the temporal profile of the photoacoustic signal. The combination of ultrasound heating and photoacoustic detection provides several advantages to flow measurement. First, both the heating and the detection maintain tight acoustic focusing in deep flow sensing. Second, the photoacoustic signal is generated from optical absorption and thus has a clean background. Third, this method does not rely on discrete scatterers or absorbers and can be potentially applied to homogenous flow media. A theoretical model is established to describe the relationship between the flow speed and the photoacoustic signal. Flow speeds up to 41 mm?s-1 have been experimentally measured in a blood phantom covered by 1.5 mm thick tissue.

8943-38, Session 6

3D high resolution photoacoustic imaging based on pure optical photoacoustic microscopy with microring resonator

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For three-dimensional imaging of optical absorbance, the existing technology of photoacoustic microscopy (PAM) has quite poor axial resolution, the tens of microns to hundreds of microns. This is despite the fact that PAM has recently achieved lateral resolutions on the order of a micron or submicron, comparable to that of optical microscopy. In this paper, a pure optical photoacoustic microscopy (POPAM) with optical rastering of a focused excitation beam and optically sensing of the photoacoustic signal using a microring resonator was developed. The bandwidth of microring resonator was promoted to provide axial resolution comparable with lateral resolutions by elaborately refining the fabrication design and improving response feature of the photodetector. With unprecedented broad bandwidth of the new microring resonator, 3D high resolution photoascoutic imaging of the tissue on the micron scale was achieved.

8943-39, Session 6

Optical resolution photoacoustic microscopy using a Blu-ray DVD pickup head

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In this study, we develop a commercial Blu-ray (405 nm) DVD pickup head based optical resolution photoacoustic microscopy (OR-PAM) for label-free micro-vascular and unstained blood smear imaging. According to the high optical absorption of the hemoglobin at 405 nm, the proposed OR-PAM has potential to be an alternative for the conventional optical microscopy in the examinations of hematological morphology for blood routine and malaria screening. The firmware of a Blu-ray DVD drive was modified to allow its pickup head to generate laser pulses with 18-ns pulse length and 0.5-nJ energy at 405 nm. To verify the in vivo imaging capability of the proposed OR-PAM, the micro-vasculature of a mouse ear was imaged without any contrast agent. The results showed that it performed better than a 200x digital optical microscope in terms of image contrast and vascular morphology. In addition, a blood smear was also imaged without any staining. The red blood cells whose mean diameter is about 7 um were well resolved and the biconcave structure could be clearly visualized. In summaries, the proposed OR-PAM has been demonstrated as a promising tool for label-free blood imaging in both small animal studies and blood examinations.

8943-40, Session 6

Slow-sound photoacoustic microscopy with enhanced axial resolution of 5.8 μm

Chi Zhang, Yong Zhou, Chiye Li, Lihong V. Wang, Washington Univ. in St. Louis (United States)

The axial resolution of photoacoustic microscopy (PAM) can be enhanced by reducing the speed of sound within the imaging region of interest. This principle was demonstrated on a previously-reported PAM system, which utilized a 125 MHz ultrasonic transducer for signal detection and the Wiener deconvolution for signal processing. With sound slowed by silicone oil immersion, we have achieved a finest axial resolution of 5.8 μm for PAM, as validated by phantom experiments. The axial resolution was also enhanced in vivo when mouse ears injected with silicone oil were imaged. After injection of silicone oil, the blood vessels were resolved more clearly. When tissue-compatible low-speed liquids become available, this approach may find applications in PAM as well as in other imaging modalities, such as photoacoustic computed tomography and ultrasound imaging.

8943-41, Session 6

Integrated confocal, two-photon, and OR-PAM (optical-resolution photoacoustic microscopy) tri-modality microscope

Bin Rao, Lijun Ma, Yu Wang, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Since Chalfie et al. first successful demonstrated the significant potential of green fluorescent protein (GFP) as a molecular fluorescent probe in 1994, confocal fluorescent microscope technology has become widely used to monitor gene expression and protein localization in living organisms. The development of genetic mutation technologies resulted in enhanced GFP (EGFP), and variously colored mutants, such as blue (BFP), cyan (CFP), yellow (YFP), and their enhanced versions (EBFP, ECFP, and EYFP). The enriched contrast mechanisms by these fluorescent proteins further promoted the wide usage of confocal microscopy. An evolution from confocal technology to two-photon microscopy provides higher contrast, lower toxicity, and deeper imaging depth due to its unique two-photon excitation approach. Even more recently developed optical-resolution photoacoustic microscopy (OR-PAM) extends microscopy technology further to image non-fluorescent chromophores, such as red blood cells. In this work, we developed a tri-modality (confocal microscopy, two-photon microscopy, and OR-PAM) for the first time. We modified a commercial FV1000MPE microscope by adding an additional pulse laser excitation channel and a photoacoustic detection mechanism. Tissue slides were prepared with plastic covers instead of glass and attached to the bottom of a petri dish with an opening at the bottom. A vacuum grease seal allowed water immersion of a transducer on top of the tissue slides. This tri-modality microscope enables potential biological applications that image both fluorescent and non-fluorescent chromophores.

8943-42, Session 7

Optical wavefront shaping-enhanced photoacoustic microscopy

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Controllable light delivery to the region of interest is essential to biomedical optical imaging methods like photoacoustic microscopy. It is, however, challenging beyond superficial depths in biological tissue (~1 mm beneath human skin) due to the strong scattering of light that scrambles the photon propagation paths. Recently, optical wavefront shaping has been proposed to modulate the incident light wavefront to compensate for the scattering-induced phase distortions, and consequentially, convey light optimally to a desired location behind or inside turbid media. To reach an optimum wavefront, a searching algorithm is usually required to optimize a feedback signal. In this work, we present our latest explorations, which use photoacoustic signals as the feedback to remotely and non-invasively guide the wavefront shaping process. Our method does not require direct optical access to the target region or the invasive embedding of fluorescence probes inside turbid media. Experimentally, we have demonstrated that diffuse light can be converged to the ultrasound focus by maximizing the amplitude of photoacoustic emissions from the intended absorbing site. Moreover, we show that wavefront-shaped light focusing can enhance existing optical imaging modalities like photoacoustic microscopy, in regard to signal-to-noise ratio, imaging depth, and potentially, resolution.

8943-43, Session 7

Impact of ultrasound focal volume changes on fluence mapping using reflection mode acousto-optics

Jacob W. Staley, Erwin Hondebrink, Wiendelt Steenbergen, Univ. Twente (Netherlands)

Fluence mapping using acousto-optics has been shown to fluence compensate photoacoustic signals in optically scattering media. Using acousto-optics for photoacoustic fluence compensation requires that the ultrasound focus, commonly referred to as the tagging volume, is unperturbed. By using a programmable commercial ultrasound system it is possible to electronically translate the tagging volume, opposed to mechanically, which increases the speed of acousto-optic fluence mapping over a spatial region. However, electronic steering alters the spatial-peak pressure within the tagging volume. Changes in the spatial-peak pressure negatively impact the ability for acousto-optics to fluence correct photoacoustic signals. Here we demonstrate how the changes in the acousto-optic tagging volume, through electronic steering of ultrasound, impact the ability to fluence compensate photoacoustics and accurately extract localized absorption coefficients. We also present the ability to compensate for the tagging volume changes by simulating the ultrasound focus from experimentally obtained data. This is achieved by compensating for the relative changes in the simulated spatial-peak temporal average acoustic intensities at different steering angles, and the corresponding relative changes in the measured speckle contrast.

8943-44, Session 7

Wave-mixing in gain media (Nd:YVO4) for acousto-optical imaging

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Acousto-optic imaging is a technique that uses the modulation of light by ultrasound to probe the local optical properties of thick scattering media at visible or near IR wavelength with a millimetric resolution. As the modulation frequency (a few MHz) is very small compared to the light frequency (several 100THz), a standard spectral filtering is hard to set up. A solution is to use detection techniques based on two or four wave mixing in nonlinear crystals to perform a wavefront adaptation of the modulated light in order to detect it on a large area single detector.

Up to now, nonlinear photorefractive crystals (such as BSO, LiNbO3 or SPS) are used to detect acousto-optical signals; after demonstrating phase conjugation in a laser medium (Nd:YVO4), we have now performed the detection of an ultrasonic burst as it travel through the multiscattering sample using a 2 wave mixing configuration. In laser media, wave mixing is achieved using the gain saturation, indeed, it is possible to spatially modulate the population inversion, and therefore the gain, by sending an interference pattern inside the crystal to record the phase information of a signal beam in a gain hologram. Then this hologram can be read to perform a wavefront adaptation in order to detect the acousto-optical modulation.

The use of a Nd:YVO4 crystal enables to detect an acousto-optical signal through several millimetres of a living tissue with a response time less than 90µs; much faster than the usual response time of a photorefractive crystal (around 10ms). This makes the gain media much more robust against speckle decorrelation in biological samples.

8943-45, Session 7

Reflection-mode time-reversed ultrasonically encoded (TRUE) optical focusing

Yuta Suzuki, Jian Wei Tay, Lihong V. Wang, Washington Univ. in St. Louis (United States)

To achieve localized light delivery beyond turbid layers, TRUE optical focusing has been previously implemented by both analog and digital devices. The digital scheme offers a higher energy gain than the analog version. In many biological applications, the reflection-mode configuration, which uses backscattered light from the sample, is more suitable than the transmission-mode configuration. Although reflection-mode analog TRUE focusing has been demonstrated, its digital implementation has not been explored. Here, we report a reflection-mode digital TRUE focusing to concentrate light through a turbid layer. Further, by simply moving the ultrasound focus, we show the system's dynamic focusing capability.

8943-46, Session 7

Recent progress in ultrasound-switchable fluorescence imaging

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Fluorescence microscopy has been widely used in biological and medical studies because it can provide micron resolution images with structural, functional and molecular contrast in cells or tissues. Unfortunately, it fails to image deep tissues due to the strong light scattering. Fluorescence diffuse optical tomography (FDOT) takes advantage of the tissue scattered light and can thus image centimeter-deep tissues, but it suffers from poor spatial resolution (a few millimeters). As a result, the majority of information in deep tissues is lost in FDOT. To reveal such essential information located inside organs away from an instrument-accessible surface, deep-tissue high-resolution fluorescence imaging is highly desired.

Ultrasound-switchable fluorescence (USF) imaging has been recently demonstrated for high-resolution deep-tissue fluorescence imaging. The promising results of the USF technique highly rely on excellent and unique USF contrast agents and a highly sensitive imaging system. For tissue imaging applications, red or near infrared (NIR) USF contrast agents are needed for avoiding significant tissue absorption and autofluorescence. Recently, we have developed more than 30 USF contrast agents and well characterized their USF performance. We will give a review of the current status of our USF contrast agents and also demonstrate their USF imaging capabilities.

8943-47, Session 7

Acousto-optic imaging using quantum memories in cryogenic rare earth ion doped crystals

Luke R. Taylor, Alexander Doronin, Igor V. Meglinski, Jevon J. Longdell, Univ. of Otago (New Zealand)

We present results on the use of quantum memories for the optical detection of ultrasound, specifically, using atomic frequency combs (AFCs) for acousto-optic phantom imaging. An efficient and highly frequency-selective filter is made using a pair of AFCs written at ~ 606 nm in Pr3+:Y2SiO5. The combs (tooth separation ? ~ 150 kHz, finesse fc ~ 2 and optical depth ?L ~ 2) are situated at the ultrasound sideband frequencies, and placed on either side of a transparency window (1-2 MHz width) allowing transmission of the carrier pulse through the filter. Only the ultrasound-modulated sidebands are stored in the quantum memory, where they are delayed in time then retrieved as a photon echo (10-20 % efficiency). Using this technique (based solely on optical pumping, without the need for external electric or magnetic fields, or requirements on the spatial quality of the beam), we have demonstrated record sideband to residual carrier discrimination using optically thin samples. We expect this high-resolution deep-tissue acousto-optic imaging technique to remain transferable to other rare-earths with shorter-lifetime primary holeburning mechanisms (e.g. Tm), for use at wavelengths deeper inside the biological transparency window. Using the described technique, we present the first results on the imaging of a phantom within a sample having similar properties to those of typical biological tissues.

8943-48, Session 7

Controlling light non-invasively in scattering media using the photoacoustic transmission-matrix

Thomas Chaigne, Ori Katz, Jérôme Gateau, A. Claude Boccara, Mathias Fink, Emmanuel Bossy, Sylvain Gigan, Institut Langevin (France)

Focusing light to the micron scale is crucial for biomedical applications such as optical microscopy and laser micro-surgery. However, the inherent inhomogeneity of biological tissues results in light scattering, which limits effective focusing to shallow depths of a few hundred micrometers. Wavefront shaping appears in the last few years as a powerful tool to restore optical focusing of a beam after propagation through such a scattering medium, but focusing inside remains a challenge. Iterative approaches using fluorescence or acousto-optic feedback optimization have been recently investigated, but these techniques require one measurement process per focus position.

We report on the use of a photoacoustic feedback signal to monitor simultaneously and non-invasively the local light intensity on a large number of endogenous absorbers inside a sample. We study the potential of a photoacoustic-based transmission matrix, connecting optical input modes and acoustic responses of the absorbers. We demonstrate its focusing ability through 0.5mm thick chicken breast tissue and investigated both one-dimensional and two-dimensional photoacoustic imaging techniques (using either a single-element transducer or a linear transducer array). Finally, we show that this transmission matrix contains much more information than the one required for focusing, and gives insight into the scattering properties of the medium. We show how this same photoacoustic transmission matrix enables to probe the memory effect as manifested inside the sample. We also exploit the singular value decomposition (SVD) of the photoacoustic transmission matrix to identify and discriminate individual absorbing targets without any a-priori knowledge of their number or positions.

8943-49, Session 8

High resolution raster-scan optoacoustic mesoscopy of genetically modified Drosophila pupae

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Optoacoutic mesoscopy aims to bridge the gap between optoacoustic microscopy and optoacoustic tomography. We have developed a setup for optoacoustic mesoscopy where we use a high frequency, high numerical aperture spherically focused ultrasound transducer, with a wide bandwidth of 25-125 MHz. The excitation is performed using a diode laser capable of >500 µJ/pulse, 1.8ns pulse width, 1.4 kHz pulse repetition rate, at a wavelength of 515 nm. The system is capable to penetrate more than 5 mm with a resolution of 7 µm axially and 30 µm transversally. Using high speed stages and scanning the transducer in a quasi-continuous mode, a field of view of 4x4 mm2 is scanned in less than 10 minutes. The system is suitable for imaging biological samples that have a diameter of 1-5mm; zebrafish, drosophila melanogaster, and thin biological samples such as the mouse ear and mouse extremities. We have used our mesoscopic setup to generate 3-dimensional images of genetically modified drosophila fly, and drosophila pupae expressing GFP from the wings, high resolution images were generated in both cases, in the fly we can see the wings, the legs, the eyes, and the shape of the body. In the pupae the outline of the pupae, the spiracles at both ends and a strong signal corresponding to the location of the future wings are observed. Although the system is currently operating in a trans-illumination mode, using miniaturized transducers will allow us to operate in an epi-illumination mode, which is suitable for more biological samples.

8943-50, Session 8

Functional pitch of a liver: fatty liver disease diagnosis with photoacoustic spectrum analysis

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To provide more information for classification and assessment of biological tissues, photoacoustic spectrum analysis (PASA) moves beyond the quantification of the intensities of the photoacoustic (PA) signals by the use of the frequency-domain power distribution, namely power spectrum, of broadband PA signals. The method of PASA quantifies the linear-fit to the power spectrum of the PA signals from a biological tissue with 3 parameters, including intercept, midband-fit and slope. Intercept and midband-fit reflect the total optical absorption of the tissue whereas slope reflects the heterogeneity of the tissue structure. Taking advantage of the optical absorption contrasts contributed by lipid and blood at 1200 and 532 nm, respectively and the heterogeneous tissue microstructure in fatty liver due to the lipid infiltration, we investigate the capability of PASA in identifying histological changes of fatty livers in mouse model. 6 and 9 pairs of normal and fatty liver tissues from rat models were examined by ex vivo experiment with a conventional rotational PA measurement system. One pair of rat models with normal and fatty livers was examined non-invasively and in situ with our recently developed ultrasound and PA parallel imaging system. The results support our hypotheses that the spectrum analysis of PA signals can provide quantitative measures of the differences between the normal and fatty liver tissues and that part of the PA power spectrum can suffice for characterization of microstructures in biological tissues. Experimental results also indicate that the vibrational absorption peak of lipid at 1200nm could facilitate fatty liver diagnosis. This work is accepted for publication in Radiology.

8943-51, Session 8

Photoacoustic microscopy of thermal diffusivity

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Thermal diffusivity is closely associated with tissue morphology and functionality such as tissue composition and cell viability. An abnormal tissue thermal diffusivity usually indicates a diseased status. Solid tumors are known to have lower thermal diffusivity than surrounding healthy tissue, due to their high cell density. Accurate measurement of tissue thermal diffusivity can be helpful for cancer diagnosis and treatment evaluation. Typically, tissue thermal diffusivity is measured by transmission-mode pump-probe methods, which require multiple lasers and complex system configurations, and are limited to optically thin samples. Here, we propose a new method for tissue thermal diffusivity measurement, based on the temperature dependence of photoacoustic signals. In our method, the tissue is repeatedly excited by short laser pulses. The residual heat of the laser excitation increases the local temperature at a rate that is determined by the thermal diffusivity, and the photoacoustic signal amplitude increases linearly with the local temperature. Here, the same laser pulses serve as both the pump and probe. A simple exponential recovery model can be used to fit for the thermal time constant, which is inversely proportional to the thermal diffusivity. To validate our method, the thermal diffusivities of different biological materials were measured with a reflection-mode optical-resolution photoacoustic microscope. The effect of laser fluence and optical focal spot size were also investigated.

8943-52, Session 8

Fourier transform photoacoustic microscopy using a multi-wavelength laser

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Functional photoacoustic microscopy (PAM) is a valuable tool for quantifying hemoglobin oxygenation within vessels. In several previous studies, quantitative oxygen saturation (SO2) measurements were taken by closely sequential pairs of pulsed laser beams. However, the generated photoacoustic (PA) signals inevitably had a time delay. In this study, we report the development of Fourier transform PAM (FT-PAM) based on a multi-wavelength continuous wave (cw) laser, that obtain the PA signal simultaneously. The FT-PAM system uses a cw argon-ion laser without an optical filter for wavelength selection in the laser cavity. We selected two output laser wavelengths (488 nm and 515 nm) by passing the beams thorough a diffraction grating. In order to distinguish between them, each beam was modulated with a different frequency around 5 MHz. The intensity modulated laser beams were recombined at the target, where they generated PA signals at once. The measured PA signals were transformed into the frequency domain by fast Fourier transform (FFT). From the frequency spectrum, we could measure each wavelength's contribution to the PA signal. We imaged blood samples with different SO2 values (oxyhemoglobin, deoxyhemoglobin, and mixed). From the measured PA signals and the optical fluence of the laser beam pair, we could calculate the SO2 level, and plot an oxygen saturation map of the blood.

8943-53, Session 8

Dual mode imaging with the multispectral opto-acoustic tomography and ultrasonically modulated optical tomography: computational modeling and experimental results

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We develop a multi-modal imaging technique utilizing a combination of Multispectral Opto-acoustic Tomography (MOT), where the ultrasound is generated by light, and Ultrasonically Modulated Optical Tomography (UMOT) that detects light modulated by ultrasound. A particular design and practical implementation of MOT and UMOT modalities into one front sensor device requires careful selection of various technical parameters, including size, geometry and relative position of light and ultrasound sources and detectors. Owing to a range of probing conditions and the complex composite structure of biological tissues, no general analytical solution exists that can accurately describe light-ultrasound interaction within biological tissues, and mimic detected optical and ultrasound signals and their changes due to structural or physiological variations. We develop a Monte Carlo (MC) model that is used to imitate light-ultrasound interaction and propagation within complex tissue-like turbid media. To achieve optimal simulation performance, we employ a recently developed parallel computing framework known as Compute Unified Device Architecture (CUDA), introduced by NVIDIA Corporation. The developed MC model is a part of the generalized computational tool for the needs of biomedical optics and biophotonics. Therefore, the current developments of MOT-UMOT experimental system, computational model, and the results of simulation in a comparison with the experiment are presented.

8943-54, Session 8

Acoustic resolution photoacoustic Doppler flowmetry: practical considerations for obtaining accurate, high resolution blood flow measurements

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An assessment has been made of various experimental factors affecting the accuracy, resolution and range of measurable flow velocities, measured using a pulsed time correlation photoacoustic Doppler technique. In this method, Doppler time shifts are quantified via cross-correlation of pairs of photoacoustic waveforms generated in moving absorbers using pairs of laser light pulses; the photoacoustic waves are detected using an ultrasound transducer. The acoustic resolution mode is employed by using the transducer focal width, rather than the large illuminated volume, to define the lateral spatial resolution. This enables penetration depths of several centimetres, unlike methods using the optical resolution mode, which limits the maximum penetration depth to approximately 1mm. However, in the acoustic resolution mode, it is difficult to detect time shifts in highly concentrated suspensions of flowing absorbers, such as red blood cell suspensions and whole blood. This challenge supposedly arises because of the lack of heterogeneity. However, we demonstrate a new signal processing method that surmounts this difficulty, and in fact provides additional information about the flow: we demonstrate the potential for mapping out the flow velocity profile across the tube. In addition, we have assessed the effect of the tube diameter, absorber concentration, and different sizes and colours of absorbing microspheres. We show that, using our signal processing scheme, it is possible to measure the flow of whole blood using a relatively low frequency detector. This important finding paves the way for application of the technique to measurements of blood flow several centimetres deep in living tissue.

8943-55, Session 8

Optical full-field holographic detection system for non-contact photoacoustic tomography

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A very fast holographic detection method for Photoacoustic Tomography is introduced. The optical excitation of embedded absorbers leads to the emission of thermoelastic pressure waves which transiently alter the surface topography, which is recorded in parallel using a high speed camera. The technique is validated by photoacoustic measurements using silicone phantoms with embedded black silicone absorbers.

As a light source for the optical detection, a Q-switched Nd:YAG-laser (532 nm, 8 ns) is used in double pulse mode with variable delay. The illuminated area in the range of about 1 cm? is imaged onto the camera, whereby a speckle pattern appears. After photoacoustic excitation the surface topography transiently alters and the speckle pattern changes in phase distribution. The phase difference between subsequent images can mathematically be evaluated with respect to geometrical surface displacement independently for each speckle.

By repetitive excitation and an increasing time delay between excitation- and detection pulse, the altering topography is sampled with several MHz using a kHz CMOS camera. The axial resolution of the method is measured to be below 3 nm. Afterwards, the data matrix is processed for reconstruction in order to obtain the absorber's location.

As a technical outlook, the acquisition of a full data set for volumetric reconstruction with a sampling rate of 10 MHz over a time period of 10 µs and an excitation- and detection rate of 1 kHz can be done within 100 ms. This is suitable for most in vivo applications and is potentially enabling non-anesthetized clinical applications.

8943-143, Session PMon

Photoacoustic spectroscopy in the monitoring of breast tumor development: a pre-clinical study

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Breast cancer is the most frequently diagnosed cancer type and its detection at an early stage can reduce the mortality rate substantially. With the aim to detect breast cancer early, by studying tumor progression in nude mice, a pulsed laser induced photoacoustic spectroscopy set up has been designed and developed. MCF-7 cells xenografts were developed using six to eight weeks old female nude mice and tumor tissues were extracted on different days (10th, 15th & 20th day) post injection and the corresponding photoacoustic spectra were recorded at 281nm excitation. A total of 144 time domain spectra were recorded from 36 animals belonging to the three time points (10th, 15th and 20th day post injection) and converted into frequency domains by fast Fourier transform (FFT) tools of the MATLAB algorithms and analyzed. The frequency patterns of the tumor masses on 10th, 15th and 20th day of tumor development showed a gradual increase in intensity at certain frequencies, 5.93 x103 Hz, 15.9 x103 Hz, 29.69 x103 Hz and 32.5 x103 Hz in the FFT patterns indicating that these frequencies were more sensitive towards tumor development. Further analysis of the data yielded a clear variation in the spectral parameters with progression of the disease suggesting that the technique may be suitable for early detection of the disease. Thus, we expect that the developed setup may be useful in assessing the different phases of tumor development which may have clinical implications.

8943-149, Session PMon

In vivo photoacoustic imaging of prostate brachytherapy seeds

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Prostate brachytherapy is a localized form of radiation therapy for prostate cancer that is administered by implanting multiple metallic radioactive seeds. The standard imaging modality for intraoperative seed localization is transrectal ultrasound, which suffers from factors such as poor acoustic contrast between seeds and tissue, reverberation artifacts, and poor detectability due to the small seed size. This work is the first to investigate the in vivo feasibility of photoacoustic imaging as a complementary alternative to transrectal ultrasound imaging. A 25 kg dog was prepared for a prostate brachytherapy procedure approved by the Johns Hopkins Animal Care and Use Committee. The dog was positioned supine with its legs and tail immobilized for unobstructed access to the perineum. A standard Nucletron stepper and needle template guide were utilized for transperineal insertion of 18G needles for seed delivery and a 1-mm core diameter optical fiber surrounded by a light diffusing sheath. The fiber, coupled to a 1064nm Nd:YAG laser, was positioned at a distance of approximately 6 mm from seeds. The seeds were coated with India ink to increase optical absorption. The average energy density measured at the cone-shaped tip of the sheath was varied from 2.9-6.0 mJ, which corresponds to energy densities of 46-95 mJ/cm^2. For these energies, the mean signal-to-noise ratios ranged 12-31 dB. Post operative CT images were acquired to confirm seed locations. Results suggest that in vivo photoacoustic imaging of brachytherapy seeds is clinically feasible within the ANSI limit for human exposure.

8943-150, Session PMon

Classification algorithm of ovarian tissue based on co-registered ultrasound and photoacoustic tomography

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Human ovarian tissue features extracted from the photoacoustic spectra data and co-registered ultrasound and photoacoustic image are used to characterize malignant vs. benign processes using a support vector machine (SVM) classifier. The center of suspicious tumor areas are located by Gaussian fitting of the mean Radon transform of photoacoustic image along 0 and 90 degrees. Normalized power spectra are calculated using the Fourier transform of the photoacoustic beamforming data across these suspicious areas, where the new features of spectral slope and 0-MHz intercept are extracted. Texture analysis based on the gray-level co-occurrence matrix of photoacoustic image along horizontal (in depth) and vertical directions is performed to extract the contrast, correlation, energy(uniformity) and homogeneity parameters. Image statistics, total photoacoustic summation, histogram fitting and max output of constructed filters with cancer or normal patterns as well as other previous used features are calculated to compose a total of 21 features. These features are extracted from 169 datasets of 19 ex vivo ovaries. Half of the cancerous and normal datasets are randomly chosen to train SVM classifier with polynomial kernel and the other half datasets are used for testing. With 50 times data resampling, for the train group, the SVM classifier gets 100% sensitivity and 100% specificity and for the testing group, the SVM classifier has achieved 91.55± 2.03% sensitivity and 88.20 ± 6.03% specificity. These results are superior to those obtained earlier by using features extracted from image statistics only.

8943-151, Session PMon

Simultaneous three-dimensional photoacoustic and confocal microscopy at optical diffraction limited resolution

Biqin Dong, Hao Li, Zhen Zhang, Siyu Chen, Kevin Zhang, Hao F. Zhang, Cheng Sun, Northwestern Univ. (United States)

Integrating photoacoustic microscopy (PAM) into multimodal optical imaging system is desired in clinical applications and scientific researches, which require complementary anatomical and functional contrasts of information for accurate diagnosis and deeper understanding of the diseases. Although PAM has been extensively studied in the field of functional biomedical imaging for its capability in noninvasive label-free imaging of biological samples in three-dimension (3D) that establishes physiologically relevant optical absorption contrast with high spatial resolution, integrating PAM to a well-established sub-micro resolution confocal microscope still remains challenging. However, the bulky physical dimension and opaque nature of traditional piezoelectric transducers become rather difficult to fit with the limited working distance of the high magnification objective and avoid obstruction to the optical imaging path. Moreover, the axial resolution of the traditional PAM is usually the orders of magnitude lower than that of the confocal microscopy due to the limited bandwidth and angular sensitivity of the ultrasound detection. Here we present an integrated system of PAM and optical confocal microscopy by using an ultra-thin transparent optical ultrasound sensor. By taking advantages of its broadband ultrasound response and minimum angular dependence, the spatial resolution reaches 1.1 ?m in lateral and less than 4 ?m in axial direction using a 20x objective lens with a field of view larger than 200 ?m and a working distance of 1 mm. Furthermore, we imaged single retinal pigment epithelium cells to demonstrate the system capabilities.

8943-152, Session PMon

Compatible optical ultrasonic sensor for commercial laser scanning microscopy

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As a noninvasive label-free imaging modality, photoacoustic microscopy (PAM) is capable of visualizing three-dimension optical absorption based contrast closely associated with physiological properties of the imaging sample. Converting a commercial laser-scanning optical microscope to be a PAM requires special ultrasonic sensors that are small in size and with minimum obstruction to the optical imaging path and maximum detectable region. Although piezoelectric transducers were widely used in traditional PAM, their bulky physical dimension and opaque nature become rather difficult to integrate them into commercial microscope system. In contrast, optical detection of ultrasound is much more desirable due to its high detecting performance and potential of miniaturization and transparency. By taking advantages of its broadband ultrasound response and minimum angular dependence, we designed ultra-thin transparent micro-ring ultrasonic sensor and integrate it to a commercial confocal microscope to achieve simultaneous multimodal imaging at sub-micro spatial resolution. Our novel optical micro-ring ultrasonic sensor is compatible with all the commercial inverted optical microscopes without any altering.

8943-153, Session PMon

Sensing of oxygen saturation of hemoglobin in ocular tissue using acoustic-resolution photoacoustic microscopy through scattering sclera

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Hemoglobin (Hb) concentration and oxygen saturation (SO2) levels are important biomarkers in the pathogenesis of ocular diseases such as macular degeneration, cataract, and glaucoma. Optical coherence

tomography (OCT) is widely used in ocular measurements, including retina structure imaging, microvasculature imaging, and, recently, for SO2 measurement. However, optical scattering in the tissue can greatly affect the detected backscattered OCT signals, and hence the SO2 measurement. Photoacoustic imaging, an emerging technology, has successfully been used in many applications such as microvasculature imaging, blood flowmetry, and functional contrast agent imaging. Recent research shows it can be applied in eye research as well, and can be integrated with the widely used OCT system. Multi-wavelength photoacoustic microscopy measures SO2 by taking advantage of the difference between the absorption spectra of the oxyhemoglobin and deoxyhemoglobin. Because absorption is the only source of photoacoustic wave, the relative sensitivity of optical absorption is 100%. Here we present the estimation of oxygen saturation of hemoglobin in ocular tissue using acoustic-resolution photoacoustic microscopy (AR-PAM). In a series of in vivo rabbit eye experiments, AR-PAM was able to image the choroid and the ciliary body through highly scattering sclera. SO2 was measured as well, under various oxygen level of breathing gas. The results show AR-PAM could measure the SO2 in specific sites of ocular tissue, and the relative change of SO2 was consistent with the oximeter measurements.

8943-154, Session PMon

Comprehensive characterization of plasmonic gold nanoparticles for photoacoustic contrast enhancement

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We performed a comprehensive photoacoustic (PA) measurement of gold nanoparticles (NPs) of various sizes and shapes in order to design exogenous agents, which enabled to enhance the contrast for molecular imaging. Aqueous solutions of gold NPs of various sizes and shapes were used as samples. We investigated the plasmonic features of the gold NPs by UV?vis?NIR extinction spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and finite difference time domain (FDTD) analysis. The PA signal, which was induced by a nanosecond laser pulse irradiation through thermoelastic process, was detected by our originally-fabricated ring-shaped ultrasound transducer consisting of a P(VdF/TrFE) film, which was arranged coaxially with an optical fiber. The time-of-flight PA signals of the aqueous solutions of gold NPs were measured and analyzed. No apparent relationship between the PA signal intensities and the absorption coefficients of the aqueous solution of gold NPs was observed. The PA signal intensities were sensitive to the shape and size of the gold NPs. The PA signal enhancement by localized surface plasmon of the polyhedral gold NPs was evaluated. We also found the characteristic photothermal stability among the different shape of gold NPs. These results suggested that it enabled to derive the optimum design of exogenous contrast agents. This study was partially supported by JST and the Collaborative Research Program of Institute for Chemical Research, Kyoto University.

8943-155, Session PMon

NIR-activated iron oxides as a new multi-functional contrast agent of photoacoustic imaging

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Iron oxide nanoparticles are commonly used contrast agents for theranostic nanomedicines because of their advantages of good biocompatibility, high stability in physiological conditions, low cytotoxicity and excellent safety record in clinical settings for human use. In this study, we develop a NIR-activated iron oxide (NIR-Fe3O4) nanoparticle as a new multi-functional contrast agent of photoacoustic (PA) imaging. Unlike traditional iron oxides, the developed NIR-Fe3O4 owns biocompatibility and optical tunability capable of providing strong optical absorption in the NIR range for PA signal generation. Its intrinsic magnetic property enables the active magnetic tumor targeting. Phantom experiments were performed to confirm the tunability of NIR-Fe3O4's optical absorption in NIR and demonstrate its magnetic targeting capability. The PA signal response of NIR-Fe3O4 as a function of concentration was also investigated. The results showed that the PA signal of NIR-Fe3O4 with OD=1.25 was 4 times of that of blood at 715 nm – the wavelength of peak absorption of the used NIR-Fe3O4. Moreover, the PA signal from NIR-Fe3O4 could be further improved by about 14 dB with magnetic targeting. Overall, we proved that the potential of the developed NIR-Fe3O4 as a good tumor targeting contrast agent of PA imaging. Future work includes verification of the photothermal stability of NIR-Fe3O4 under pulsed laser and the performance of photothermal therapy.

8943-156, Session PMon

Magnetomotive photoacoustic imaging with magnetic-conjugated-polymer nanoagent

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Magnetomotive photoacoustic (mmPA) imaging has shown enhanced specificity compared to conventional PA imaging by magnetically manipulating cells or molecules targeted with coupled magnetic-optically-absorptive particles to suppress background signals. However, commonly used metallic nanoparticles (e.g., gold nanoparticle) are not biodegradable and thus difficult for clinical translation. A conjugated–polymer nanoparticle (CPP) with magnetic property is presented in this study as a new type of contrast agent. Compared to conventional metallic nanoagents, this new probe exhibits lower cytotoxicity and higher photostability, but maintains size and optical wavelength tunability. The efficacy of this composite agent, an iron oxide and conjugated-polymer core-shell nanoparticle (MCPP), for mmPA imaging was demonstrated in an 8% polyvinyl alcohol phantom containing two 2-mm diameter cylindrical inclusions. The first contains 0.33 nM of MCPPs with an absorption coefficient of about 0.5 cm-1 at 800 nm, and the other contains CPPs with the same optical absorption, representing background to be suppressed. An electromagnet placed below the phantom delivered 0.4-Tesla pulses, and a 15-MHz single element transducer was scanned to recorded PA images with laser illumination (9.9 mJ/cm2) from the top. A weighting map, based on the magnetically induced displacement derived by motion tracking in each image pixel, was created to multiply the original PA image. Results show that the magnetism-insensitive inclusion was suppressed by more than 30 dB while the MCPP inclusion remained unchanged, demonstrating contrast enhancement using this new generation of clinically-translatable nanoagents in mmPA imaging for widespread animal studies and clinical applications.

8943-157, Session PMon

Co-registered spectral photoacoustic tomography and ultrasonography of breast cancer

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Many breast cancer patients receive neoadjuvant treatment to reduce tumor size and enable breast conserving therapy. Most imaging methods used to monitor response to neoadjuvant chemotherapy or hormone therapy depend on overall gross tumor morphology and size measurements, which may not be sensitive or specific, despite tumor response on a cellular level. A more sensitive and specific method of detecting response to therapy might allow earlier adjustments in treatment, and thus, result in better outcomes while avoiding unnecessary morbidity. We developed an imaging system that combines spectral photoacoustic tomography and ultrasonography to predict breast neoadjuvant therapeutic response based on blood volume and blood oxygenation contrast. The system consists of a tunable dye laser pumped by a Nd:YAG laser, a commercial ultrasound imaging system (Philips iU22), and a multichannel data acquisition system which displays co-registered photoacoustic and ultrasound images in real time. Early studies demonstrate functional imaging capabilities, such as oxygen saturation and total concentration of hemoglobin, in addition to ultrasonography of tumor morphology. Further study is needed to determine if co-registered photoacoustic tomography and ultrasonography system may provide an accurate tool to assess treatment efficacy by monitoring tumor response in vivo.

8943-158, Session PMon

Photoacoustic tomography using a fibre laser and coded excitation schemes

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Fibre lasers have the advantage of being compact, robust and efficient compared to traditional excitation sources used for photoacoustic tomography (e.g. Q-switched Nd:YAG pumped OPO or dye systems). So far they have however been rarely used for photoacoustic tomography imaging due to their relatively low peak power (kW) compared to that provided by Q-switched lasers (MW). However, the ability to arbitrarily modulate the temporal shape of the excitation pulse, allows for pulse compression techniques similar to those used in radar, sonar and ultrasound to be implemented. For peak power limited sources such as fiber lasers, these pulse compression techniques have the potential to increase the SNR of the photoacoustic signals by distributing the excitation energy over an extended period of time. In addition the high pulse repetition frequencies (>100Hz) of the fibre laser would allow for the imaging time to be reduced.

Three pulse compressions schemes have here been investigated, Barker code, Golay code and Maximum Length Sequences (MLS). For each scheme, simulations and experimental work was undertaken to determine the conditions that provide a net SNR gain given the competing constraints of safe laser exposure, laser peak power limit and acquisition speed requirements. The experimental work was undertaken using a custom designed fibre laser. This excitation source was also combined with a Fabry Perot photoacoustic scanning system to provide 3D images of tissue mimicking phantoms as well as the vasculature of the palm of the hand. This study demonstrated that fibre laser sources could potentially be a suitable alternative to Q-switched lasers for photoacoustic tomographic imaging of superficial blood vessels.

8943-159, Session PMon

Gold nanocages and phase-change materials for photoacoustic imaging and controlled drug release by high-intensity focused ultrasound in vivo

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This paper describes a new theranostic system that both enhances the contrast of photoacoustic imaging and controls the release of drugs by high-intensity focused ultrasound (HIFU). The fabrication of this system involves filling the hollow interiors of gold nanocages with a phase-change material (PCM) such as 1-tetradecanol, which has a melting point of 37–40°C. The PCM can be premixed and thus loaded with doxorubicin (DOX) or other drugs. AuNCs with a localized surface plasmon resonance peak at ~835 nm achieve the best loading efficiency for DOX. When exposed to direct heating or HIFU, the PCM melts and escapes from the nanocages, concurrently releasing the encapsulated drugs into the surrounding medium. We can control the release profile by varying the pH value of the surrounding medium, the power of HIFU, and the duration of exposure to HIFU. The feasibility of the theranostic system was further demonstrated by in vitro cell viability test and in vivo tumor treatment experiments using MDA-MB-435 cells.

8943-161, Session PMon

Dual plasmonic gold nanoparticles for multispectral photoacoustic imaging applications

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Nanoparticle contrast agents for molecular targeted imaging have widespread interest for diagnostic applications with cellular resolution, specificity and selectivity for visualization and assessment of various disease processes. Of particular interest is gold nanoparticle owing to its tunability of the surface plasmon resonance (SPR) and its relative inertness. Here we present the synthesis of anisotropic multi-branched star shaped gold nanoparticles exhibiting dual-band plasmon absorption peaks and its application as a contrast agent for multispectral photoacoustic imaging. The transverse plasmon absorption peak of the synthesised dual plasmonic gold nanostar (DPGNS) was around 700 nm and that of longitudinal plasmon absorption in the longer wavelength region around 1100-1250 nm. Unlike most reported PA contrast agent with surface plasmon absorption in the range of 700 to 800 nm showing moderate tissue penetration, 1100-1250 nm range lies in the farther region of the optical window of biological tissue where scattering and the intrinsic optical extinction of biological chromophores is at its minimum. We present an in vivo proof of principle demonstration of DPGNS as contrast agent for multispectral photoacoustic imaging. Our results show that DPGNS are promising for PA imaging with extended-depth imaging applications.

8943-162, Session PMon

Overcoming in vivo speckle decorrelation in acousto-optics using tandem optical pulses

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Acousto-optic imaging is a promising image modality that is sensitive for optical properties. In reflection mode it is even possible to map the fluence distribution. Unfortunately in-vivo applications remain challenging due to fast speckle decorrelation (<1ms). We present an ultra fast acousto-optic imaging system that uses a ns pulsed laser system with coherent light. The pulses are converted in a tandem pulse with a peak separation of <100 ns. This allows for probing one point in the sample within the speckle decorrelation time of tissue and even liquid samples. We convert the pulse by splitting the beam, delaying one path and combining them. The challenge is the matching of the wavefronts that should have a high overlap integral, such that the resulting speckle patterns are as identical as possible. When ultrasound is introduced the otherwise identical speckle pattens on the camera are now different. The camera integrates both patterns what results in a speckle pattern with a lower contrast. The bigger the contrast difference the more light is tagged. The amount of tagged light is our signal that can be spatially mapped by scanning the ultrasound probe over the sample. We show the initial results of our method on very fast decorrelating samples.

8943-163, Session PMon

Probing confined and unconfined hemoglobin molecules with photoacoustics

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Hemoglobin molecules, confined within red blood cell (RBC) under normal physiological conditions, become unconfined due to the breakdown of cell membrane. This is known as hemolysis and is associated with various circulatory disorders. This work experimentally investigates the role of confinement of hemoglobin on photoacoustic (PA) emission.

In vitro experiments were conducted with porcine RBCs at 532 and 1064 nm at various fluences. Fluence was varied between 8 to 21 mJ/cm2/ pulse for 532 nm and 353 to 643 mJ/cm2/pulse for 1064 nm. The PA signals from suspended RBCs (SRBCs) and hemolyzed RBCs (HRBCs) at hematocrits ranging from 10 to 60% were recorded using a needle hydrophone (1 mm diameter).

The PA amplitude for both the samples increased linearly for both optical wavelengths as the hematocrit increased. The SRBCs and the HRBCs were found to generate the PA signals of comparable amplitudes at 532 nm. This trend was observed at all fluences. Nevertheless, the SRBCs produced the PA signal of higher amplitude than the HRBCs at each hematocrit for 1064 nm. The difference increased with increasing hematocrit for each fluence level.

The absorption of light by the hemoglobin molecules is similar for the SRBCS and the HRBCs. However, scattering and internal reflections of photons at the cellular boundaries might have contributed to such an optical wavelength dependent PA behavior for the SRBCs with respect to the HRBCs. This observation may open up a possibility to detect hemolysis with PAs.

8943-164, Session PMon

Polyimide-etalon all-optical ultrasound transducer for high frequency applications

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In this work we have developed an all-optical high frequency ultrasound transducer consisting of a UV-absorbing polyimide film integrated into an etalon receiver. For high sensitivity, a dielectric stack having high NIR reflectivity was chosen for the first mirror. The mirror also has high UV transmittance allowing for polyimide as the etalon medium thereby maximizing the damage threshold and acoustic output. 2.5 µm of polyimide (HD Microsys. PI-2555) was spin-coated onto a 3 mm fused silica substrate. A 4 µm dielectric stack having a transmittance of 93 % at 355 nm and 1.8% at 1600 nm was then deposited (Evaporated Coatings, Inc.). For the etalon medium, a 5.5 µm layer of polyimide (HD Microsys. PI-2525) having greater than 99.9 % absorption at 355 nm was spin-coated followed by a 50 nm Au mirror. A 355 nm Nd:YAG pulsed laser (pulsewidth: 13 ns, energy: 0.5 uJ) for ultrasound generation and a tunable NIR CW laser for detection were both focused onto the device with a spot diameter of approx. 150 µm and 35 µm, respectively. The etalon had a finesse of 70 resulting in an Noise-Equivalent Pressure of 2.1 kPa over a bandwidth of 2.5-50 MHz or 0.3 Pa/Sqrt(Hz). A pressure of 270 kPa was measured at the device surface, and the -3 dB lower cutoff frequency of the emission was 26.9 MHz extending beyond 60 MHz (limited by calibration data). The pulse-echo had a center frequency of 35 MHz with a -6 dB bandwidth of 49 MHz or 140 %.

8943-165, Session PMon

Theoretical and experimental investigation of multispectral photoacoustic osteoporosis detection method

Idan Steinberg, Isreal Gannot, Avishay Eyal, Tel Aviv Univ. (Israel)

Osteoporosis is widespread, has a catastrophic impact on patients lives and overwhelming related healthcare costs. Recently, we proposed a multispectral photoacoustic technique for early detection of osteoporosis. Such technique has great advantages over pure ultrasonic or optical methods as it allows the deduction of both bone functionality from the bone absorption spectrum and bone resistance to fracture from the characteristics of the ultrasound propagation. We demonstrated the propagation of multiple acoustic modes in animal bones in-vitro.

To further investigate the underlying mechanisms of photoacoustic generation and propagation in bones, we are fabricating a controlled dual optical and acoustical bone mimicking phantoms. These are made of Delrin® outer cortex filled with blood and fat. Phantoms which simulate both naïve and Osteoporotic bones were investigated. Such samples are simple enough to allow theoretical modeling yet captures the essence of light scattering in the cortex, photoacoustic generation in the bone interior and propagation of multiple acoustic modes in the cortex.

Theoretical investigation was performed using a Monte Carlo simulation of the photon paths, followed by an acoustic finite element simulation. The experimental investigation was based on a directly modulated laser diodes system in the near-IR spectral range. The excitation position was scanned along proximal end of the bone while the phase and amplitude of the acoustic modes traveling along the bone shaft were measured near the distal end of the bone.

The comparison of theoretical and experimental results is discussed. The relations between photoacoustic observables and parameters of clinically significance are presented.

8943-166, Session PMon

Detecting occlusion inside a ventricular catheter using photoacoustic imaging through skull

Behnoosh Tavakoli, Xiaoyu Gua, Jin U. Kang, Russell H. Taylor, Johns Hopkins Univ. (United States); Emad M. Boctor, Johns Hopkins Outpatient Ctr. (United States)

Hydrocephalus is a medical condition caused by accumulation of excessive cerebrospinal fluid (CSF) in the ventricular space that creates increased pressure on the brain. It is one of the most common birth defects in children with 1 in 500 occurrence rate. The most common treatment for hydrocephalus is placement of a CSF shunt to divert excess CSF to a re-absorption site and therefore regulate intracranial pressure. Unfortunately these shunts have an unacceptably high incidence of occlusions due to in-grown tissues that block CSF flow. Failure rates are estimated to be ~40% in the first year and ~80% within 10 years.

We have studied a method to image the occlusion inside the shunt through the skull. In this approach the pulsed laser light coupled to the optical fiber illuminate the occluding tissue inside the catheter and an external ultrasound transducer is applied to detect the generated photoacoustic signal. The feasibility of this method is investigated using a phantom made of ovis aries brain tissue and adult human skull. We were able to image the target inside the shunt located 20mm deep inside the brain through about 4mm thick skull bone. This study could lead to the development of a simple, safe and non-invasive device for percutaneous restoration of patency to occluded shunts. This will eliminate the need of the surgical replacement of the occluded catheters which expose the patients to risks including hemorrhage and brain injury.

8943-167, Session PMon

Optical focusing into scattering media by digital ultrasonic encoding (DUE)

Jian Wei Tay, Puxiang Lai, Yuta Suzuki, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Delivering light to a target region within scattering media is a much sought-after goal in biomedical optics. In optical therapy and manipulation, a sufficient photon density in the targeted region determines both efficacy and specificity, while for imaging, the optical spot size determines the resolution. However, using conventional lenses, optical focusing is limited to one transport mean free path, as scattering causes the wavefront to no longer add up in phase. There has been growing interest in overcoming this limit, using techniques such as optical phase conjugation or wavefront shaping to compensate for the scattering. In practice, wavefront shaping provides a more robust optical system which is less sensitive to optical alignment. Here, the phase of the incident light is spatially tailored using a phase-shifting array to compensate for the scattering. The challenge, then, is to optimize the phase pattern on the modulator. In the past, visible particles were used as targets to generate feedback to optimization algorithms. However, embedding these particles is invasive, and light delivery is limited to fixed positions. Here, we demonstrate a method for non-invasive and dynamic focusing, by using ultrasonically encoded light as feedback. This technique has broad biomedical applications, such as in photodynamic therapy, optical manipulation, and optogenetics.

8943-168, Session PMon

Calibration-free structured-illumination photoacoustic flowgraphy of transverse flow

Junjie Yao, Rebecca Gilson, Lidai Wang, Konstantin I. Maslov, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Blood flow measurement by photoacoustic (PA) imaging is gaining interest, as it takes advantage of weak acoustic scattering and high optical absorption contrast. Optical-resolution photoacoustic microscopy (OR-PAM), a major implementation of PA imaging, focuses on capillary level spatial resolution within the optical diffusion limit (~1 mm in tissue). OR-PAM has been used to measure transverse blood flow speed based on bandwidth broadening, while the flow direction can be measured using bidirectional scanning. However, a phantom calibration is needed to account for particle size. Moreover, tissue scattering reduces the optical focusing capability. The enlarged focal spot in deeper tissue results in an underestimation of flow speeds. Here, we propose a calibration-free photoacoustic (PA) method for transverse flow measurements. In this method, a pulsed periodically structured (i.e., grating patterned) optical beam is used to illuminate flowing absorptive particles in an optically scattering medium. The PA signal amplitudes measured over consecutive laser pulses carry an imprint of the illumination structure. The modulation frequency of the imprint is proportional to the component of the flow speed projected onto the normal axis of the illumination pattern. This method can tolerate high particle density, and is insensitive to the particle size, thus it is calibration-free. Bovine blood and microsphere phantoms were used to validate the proposed method. A measurement accuracy of 0.05 mm/s was achieved. Blood flow in a mouse ear was measured in vivo as well.

8943-169, Session PMon

Accuracy of retinal oximetry: a Monte Carlo investigation

Wenzhong Liu, Northwestern Univ. (United States); Shuliang Jiao, Florida International Univ. (United States); Hao F. Zhang, Northwestern Univ. (United States)

Retinal hemoglobin oxygen saturation (sO2) level is believed to be associated with the pathophysiology of several eye diseases, such as diabetic retinopathy, retinopathy of prematurity, photoreceptor degeneration and so on. Methods to properly measure retinal sO2 have been investigated for decades, with fundus photography being the most established method; however, the accuracy of fundus photography in retinal oximetry is still considered to be limited. Recently developed photoacoustic technique is demonstrated to be able to quantify sO2 precisely, who's application on retinal sO2 , yet awaits being reported. Thus, knowing performance theoretically of both fundus camera and photoacoustic ophthalmoscope (PAOM) under various environments is important for retinal sO2 detection. In present study, we applied the Monte Carlo to simulate, and examine how the accuracy of retinal oximetry is affected by local parameters. Fundus photography was simulated in a multilayer retinal model, in which a single vessel segment with 70% sO2 was embedded, at six optical wavelengths. Then, 200 million photons were traced in each simulation to ensure statistically stable results. The optical reflectance and energy deposit were recorded to measure sO2 using both the fundus photography and PAOM. By varying the vessel diameter and melanin concentration in the retinal pigment epithelium, the relative error of sO2 measurement in fundus photography increased with increasing vessel diameter and melanin concentration; in comparison, the sO2 measurement was insensitive to these two parameters in PAOM.

8943-170, Session PMon

Propagation of photoacoustic shock waves

Xinmai Yang, The Univ. of Kansas (United States); Janggun Jo, KU Bioengineering Research Ctr. (United States)

Photoacoustic imaging has been recently developed for biomedical imaging. This imaging technique is based on the photoacoustic effect, which includes a process involving the absorption of photons, the subsequent thermal expansion, and propagation of photoacoustic waves. The propagation of photoacoustic waves has been modeled by using linear acoustic theories although the generated photoacoustic waves are naturally shock waves. In this work, the propagation of photoacoustic shock waves are studied by using nonlinear acoustic wave solutions based on Hugoniot's shock relation combining Earnshaw solution with Poisson solution. The non-linear solution is compared with the existing linear solution, and found that there is a discrepancy between the two solutions although the difference may not be significant.

8943-172, Session PMon

Dependence of photoacoustic signal generation on the transducer and source type

Carlos A. Bravo Miranda, Arturo González Vega, Gerardo Gutiérrez Juárez, Univ. de Guanajuato (Mexico)

The influence of size, geometry, temporal response of finite sensors and source geometry on the detected photoacoustic pressure is explored. The transducer was simulated by a mesh of point-like sensors connected in parallel, which implies that adequate modeling of pressure depends on the discretization size of the sensing surface. Considering source discretization we simulated the point-like, line, cylindrical and spherical source geometries, located at certain position over an axis in the center of the sensor perpendicular to the sensing surface. In order to simulate the photoacoustic signal, the simulated pressure was convolved with the impulse response of two kind of commercial sensors: a low frequency transducer (3.5 MHz) and a high frequency transducer (125 MHz). Taking a fixed coordinate system we investigated the signal variations when the translational and rotational degrees of freedom were modified, according with the possible variations in experimental scenarios. We found that simulated pressure from the proposed approach for the finite sensor clearly differs from the point-like detection model.

8943-173, Session PMon

Iterative reconstruction algorithms for photoacoustic tomography using time reversal

Benjamin T. Cox, Univ. College London (United Kingdom); Leonid Kunyansky, The Univ. of Arizona (United States); Robert J. Ellwood, Bradley E. Treeby, Simon R. Arridge, Univ. College London (United Kingdom)

Time reversal photoacoustic image reconstruction has found widespread use as a method for forming images from experimental measurements. It is intuitive, simple to implement, makes fewer assumptions than many other approaches, and can be straightforwardly adapted to account for the effects of acoustic absorption. However, it is no longer exact when the measurement surface does not completely surround the object or when there is reverberation due to reflections from the sensors or from a non-uniform acoustic medium, and there is no obvious way in which to include regularisation (other than smoothing the data). Iterative approaches using time reversal reconstruction can overcome these concerns. In this talk, iterations using limited view data in free-space will be shown to recover the image exactly where the visibility region is satisfied using the prior knowledge that the initial particle velocity is zero. Furthermore, the iterative reconstruction procedure proposed by Stefanov and Uhlmann [Inverse Problems, 25, 075011, 2009] will be applied to recover an image from reverberant data. Finally, an iterative scheme that applies total variation regularisation will be described. This is more relevant to the vessel-type images often obtained in photoacoustics than simple smoothing regularisation such as Tikhonov. The acoustic inversion in photoacoustic imaging is sometimes considered solved, but this talk will show that there are many interesting and practical improvements that can be made to improve photoacoustic images obtained from real data sets by iterating.

8943-174, Session PMon

Three-dimensional modeling of the transducer shape in acoustic resolution optoacoustic microscopy

Xosé Luis Deán-Ben, Hector Estrada, Moritz Kneipp, Jake B. Turner, Daniel Razansky, Helmholtz Zentrum München GmbH (Germany)

Acoustic resolution optoacoustic (photoacoustic) microscopy is a powerful modality allowing imaging morphology and function at depths up to a few centimeters in biological tissues. This optoacoustic configuration is based on a spherically-focused ultrasonic transducer raster scanned on an accessible side of the sample to be imaged. Volumetric images can then be formed by stacking up the recorded time-resolved signals at the measuring locations. However, the focusing capacity of a spherically-focused transducer depends on its aperture and on the acoustic frequency of the collected signals, leading to artefacts in the images if a simplistic reconstruction approach is employed. In this work, we make use of a three-dimensional model-based reconstruction procedure developed in our group to account for the shape of spherically focused transducers in acoustic resolution optoacoustic microscopy set-ups. By discretizing the transducer shape to a set of subsensors, the resulting model incorporates the frequency-dependent transducer sensitivity to the acquisition of broadband optoacoustic signals. The inversion of the full model incorporating the effects of the transducer shape is then performed iteratively. The retrieved results indicate a good performance of the method for absorbers of different sizes emitting optoacoustic waves with different frequency spectra.

8943-175, Session PMon

S-sequence patterned illumination for fixed-point multiple illumination photoacoustic tomography

Tyler J. Harrison, Peng Shao, Roger J Zemp, Univ. of Alberta (Canada)

Fixed-point iteration shows promise for quantitative reconstruction of optical absorption in photoacoustic tomography. However, there are issues that prevent the technique from being practical including: non-uniqueness of scattering and absorption profiles, divergence with over-iteration, and sensitivity to noise. Multiple illumination has been proposed to deal with the first problem, and may help with the second. The issue of noise may be balanced out by increasing the regularization parameter at the expense of the exactness of the reconstruction. In a multiple-illumination setup with a circular geometry where fluence is abundant, using a patterned illumination with a decoding step may provide an alternative which will boost SNR. We present a simple sequence of patterned illuminations based on an S-sequence that serves to improve SNR. While the forward model of the iterative method may be applied directly to the patterned excitations, including the decoding step improves SNR in an individual image by a factor equal to the size of the S-sequence, thus greatly improving convergence for a given value of regularization and SNR. For example, with 15 illuminations, the 60dB case with S-sequence patterned illuminations gives similar simulated performance to the 70dB case with single-source illuminations. This technique will allow the application of fixed-point iteration techniques in a broader range of SNR conditions without resorting to averaging.

8943-176, Session PMon

Image reconstruction in ultrasound-modulated optical tomography

Terence S. Leung, Samuel Powell, Univ. College London (United Kingdom)

Light is highly scattered in a turbid medium, causing most of its spatial information to be lost. Therefore, optical data cannot be used directly to form an image of the medium's optical properties. To do so, image reconstruction algorithms have been developed in the diffuse optical tomography (DOT) community, though the spatial resolution remains

limited due to the underlying diffusion process. Ultrasound-modulated optical tomography (UOT) aims to improve the spatial resolution by incorporating focused ultrasound which "tags" the diffuse light in known locations. Most UOT images in the literature are formed by directly mapping the detected UOT signals, measured by techniques such as photorefractive crystal, spectral hole burning, speckle contrast and digital autocorrelator, to a grid of ultrasound scan locations. However, these images account for both the optical and acoustic properties of the medium and do not have a unit specifically related to the optical properties, making their interpretation difficult. To resolve this problem, algorithms are currently being developed to invert UOT data to reconstruct the spatial distribution of optical properties, as in the case of DOT. In this work, we introduce one such algorithm which employs a correlation diffusion equation as a forward model to predict the measured autocorrelation function. Images of the perturbed absorption coefficients, independent of the acoustic properties, are reconstructed using both UOT and DOT data obtained from phantom experiments. The results show an improved spatial resolution in the UOT images in comparison to its DOT counterpart.

8943-177, Session PMon

FPGA implementation of undecimated wavelet transform denoising and Fourier deconvolution for photoacoustic microscopy

Ryan T. Maxson, Scott P. Mattison, Brian E. Applegate, Texas A&M Univ. (United States)

Photoacoustic microscopy (PAM) is a rapidly growing hybrid biomedical imaging modality which, by combining optical excitation and acoustic detection pathways, exploits the optical absorption properties of various biological chromophores along with the desired weak acoustic scattering in tissue. Due to the bipolar nature of the detected pressure wave along with the presence of additive noise, a reconstruction scheme is required to ascertain morphologically correct results. These methods are typically performed post-acquisition, limiting PAM's scope of real-time applications. We demonstrate the use of a Field Programmable Gate Array (FPGA) for real-time signal acquisition and enveloping of PAM signals through denoising and deconvolution. We first perform Fourier Domain deconvolution to correct the bi-polar photoacoustic signal and reduce blurring introduced by the imaging system. Following deconvolution, we demonstrate the implementation of an undecimated wavelet transform (UWT) to remove ringing artifacts and suppress undesired frequencies. A wavelet basis is used that simplifies the reconstruction process so that the original signal can be built from the addition of the obtained coefficients. Due to the reconfigurable architecture of the FPGA, both of these processes can be performed in parallel with data collection enabling real-time processing following an initial throughput latency. Through this technique, we demonstrate an SNR improvement of 9.5 dB and a 15% improvement in axial resolution with parallel processing.

8943-178, Session PMon

Co-registered high frequency, high frame rate photoacoustic imaging for visualizing intra-cardiac blood flow of zebrafish

Jinhyoung Park, Volcano Corp. (United States) and Univ. of Southern California (United States); Thomas M. Cummins, The Univ. of Southern California (United States); Michael Harrison, Children's Hospital Los Angeles (United States); Ching-Ling Lien, Children's Hospital Los Angeles (United States) and Univ. of Southern California (United States); K. Kirk Shung, Qifa Zhou, The Univ. of Southern California (United States)

The zebrafish heart is well known for its regenerative capability and is used for an animal model for the studies on human heart diseases. Physical recoveries of the fish heart has been visualized with histology method which needs to sacrifice the fish, and the further follow-ups for the same fish is impossible. High frequency ultrasound imaging techniques are employed for imaging the clot disappearance, but the non-invasive imaging of the intracardiac blood flow, which can be a key indicator of functional recovery, has not been demonstrated.

In this paper, a technique on high frame rate(28fps), high frequency coregistered ultrasound and photoacoustic imaging for visualizing zebrafish heart blood flow was demonstrated. This approach was achieved with a 40MHz light weight(0.38g) ring-type transducer, serving as the ultrasound transmitter and receiver, to allow an optic fiber, coupled with a 532nm laser, to be inserted into the hole. Ultrasound and laser pulses are alternately transmitted, and both ultrasound and photoacoustic echoes are received by the same transducer. From the wire target study, axial resolutions of 38?m and 42?m were obtained for ultrasound and photoacoustic imaging, respectively. Carbon nanotubes were utilized as contrast agents to increase the flow signal level by 20dB in phantom studies, and zebrafish heart blood flow was successfully observed. The blood flow signals which are more dynamic than the signals from tissue regions are separated by subtracting a frame from the previous.

8943-179, Session PMon

Optoacoustic detection and monitoring of blast-induced intracranial hematomas in rats

Andrey Petrov, Karon E. Wynne, Donald S. Prough M.D., Douglas S. Dewitt, Yuriy Y. Petrov, Irene Y. Petrov, Margaret A. Parsley, Rinat O. Esenaliev, The Univ. of Texas Medical Branch (United States)

Patients with acute intracranial hematomas often require surgical drainage within the first four hours after traumatic brain injury (TBI) to avoid death or severe neurologic disability. CT and MRI permit rapid, noninvasive diagnosis of hematomas, but can be used only at a major health-care facility. At present, there is no device for noninvasive detection and characterization of hematomas in pre-hospital settings. We proposed to use an optoacoustic technique for rapid, noninvasive diagnosis and monitoring of hematomas, including intracranial hematomas. Unlike bulky CT and MR equipment, an optoacoustic system can be small and easily transported in an emergency vehicle. In this study we used a specially-designed blast device to inflict TBI in rats. A near-infrared OPO-based optoacoustic system developed for hematoma diagnosis and for blood oxygenation monitoring in the superior sagittal sinus (SSS) in small animals was used in the study. Optoacoustic signals recorded simultaneously from the SSS and hematomas allowed for measurements of their oxygenations. The presence of hematomas was confirmed after the experiment in gross pictures of the exposed brains. After blast the hematoma signal and oxygenation increased, while SSS oxygenation decreased due to the blast-induced TBI. The increase of the oxygenation in fresh hematomas may be explained by the leakage of blood from arteries which have higher blood pressure compared to that of veins. These results indicate that the optoacoustic technique can be used for early diagnosis of hematomas and may provide important information for improving outcomes in patients with TBI or stroke (both hemorrhagic and ischemic).

8943-180, Session PMon

Three-dimensional arbitrary trajectory photoacoustic microscopy

Chenghung Yeh, Washington Univ. in St. Louis (United States); Brian T. Soetikno, Washington Univ. in St Louis (United States);

Song Hu, Washington Univ. in St Louis (United States) and Univ. of Virginia (United States); Konstantin I. Maslov, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Optical-resolution photoacoustic microscopy (OR-PAM) is capable of functional, structural, and molecular imaging of microvasculature in vivo. Recently developed, random-access optical-resolution photoacoustic microscopy allows users to scan arbitrarily shaped regions of interest, but its imaging area is limited to the focal spot of the transducer. Contour-scanning photoacoustic microscopy can maintain a good signal-to-noise ratio (SNR) along the depth direction, although the acquired image contains extraneous information.

We have developed three-dimensional arbitrary trajectory (3-DAT) scanning. This technique can rapidly image a vessel of interest over a large area and maintain a high SNR along the depth direction. First, a global map is acquired, and the vessel of interest is selected. Then, the selected path guides the three mechanical motors for 3-DAT scanning. The 3-DAT scan was demonstrated by imaging a human hair within an area of 4x2 mm2.The frame rate was up to 10 times faster than a global scan. Furthermore, we showed that sO2 and blood flow can be measured simultaneously for the quantification of local metabolic rate of oxygen (MRO2) in a mouse ear in vivo. We also observed sO2 dynamics in response to switching physiological states between systemic hyperoxia and hypoxia. The 3-DAT scan holds great potential for studying stroke and epileptic seizure.

8943-181, Session PMon

Optoacoustic detection of Kudoa thyrsites infection in Atlantic Salmon

Michelle Patterson, Univ. of Prince Edward Island (Canada); Jonathan Horrocks, Bruno Ouimet, Sophie St. Hilaire, Atlantic Veterinary College (Canada); William M. Whelan, Univ. of Prince Edward Island (Canada)

Optoacoustic (OA) imaging and spectroscopy is being investigated for the detection of Kudoa thyrsites infection in Atlantic Salmon. The parasite does not harm the fish, but 1-2 days post mortem, the parasite releases enzymes that induce muscle autolysis, which acts to liquefy the muscle tissues and, hence, reduces marketability. Tissue infections are confirmed typically by microscopy (wet mount) or histology. There is a need for a non-destructive screening approach that would enable the identification of infected fish early in the production process in order to minimize economic losses.

In this pilot study, we utilize a backward-mode optoacoustic imaging system consisting of a Nd:YAG pumped Ti:Sapphire laser, 10 Hz repetition rate and 6 ns pulse, and an 8 element annular transducer array with a central frequency of 5 MHz (ImagioTM , Seno Medical Instruments Inc., San Antonio, TX). Healthy and confirmed infected salmon muscle samples were investigated. The OA signals were acquired for 1064 nm illumination and a surface irradiance of 10 mJ/cm2. Signals from a 2 mm cube region of interest at the tissue surface were analyzed based on amplitude and radio frequency (RF) spectral components (midband and slope) obtained from a linear fit of the calibrated optoacoustic spectra.

The infected and normal samples were visually indistinguishable. However, the infected samples exhibited an average 2.7 fold decrease in OA signal amplitude compared to the normal samples. This signal decrease is consistent with surface myoliquefaction of the muscle fibers. The RF spectrum analysis showed a 17% increase in spectral slope for infected samples compared to normal samples. In addition, OA results are compared to high frequency ultrasound (Vevo TM, Visualsonics). Overall, optoacoustics appear to be superior for distinguishing normal from K. thyrsites infected Atlantic salmon.

8943-182, Session PMon

Effectiveness of the far-field approximation for transducer modeling in photoacoustic computed tomography

Kenji Mitsuhashi, Kun Wang, Mark A. Anastasio, Washington Univ. in St. Louis (United States)

Photoacoustic computed tomography (PACT), also known as optoacoustic tomography, is a hybrid imaging modality that combines the rich contrast of optical imaging methods with the deep penetration and high spatial resolution of ultrasound imaging methods. When ultrasonic transducers with large detecting areas and/or compact measurement geometries are employed, the spatial resolution of images reconstructed in PACT can be significantly degraded. To mitigate such effects, an imaging model was proposed that compensates for the spatial impulse responses (SIRs) of the transducers by use of a far-field approximation. While the far-field-based imaging model possesses attractive computational characteristics, there remains an important need to clarify its domain of validity within the context of practical three-dimensional (3D) PACT imaging system configurations. In this work, computer-simulation studies are conducted that demonstrate the far-field-based imaging model is highly accurate for a wide-range of 3D PACT imaging configurations that employ flat transducers. For use in special cases where the far-field approximation is violated, an extension of the far-field-based imaging model is proposed that divides the transducer surface into a small number of patches that are each accurately described by use of the far-field approximation.

8943-183, Session PMon

Differentiating fatty and non-fatty tissue using photoacoustic imaging

Leo L. Pan, Robert Rohling, Purang Abolmaesumi, Septimiu Salcudean, Shuo Tang, The Univ. of British Columbia (Canada)

In this paper, we demonstrate a temporal-domain intensity-based photoacoustic (PA) imaging method that can differentiate between fatty and non-fatty tissues. PA pressure intensity is partly dependent on the tissue's speed of sound, which increases as temperature increases in non-fatty tissue and decreases in fatty tissue. Therefore, by introducing a temperature change in the tissue and subsequently monitoring the change of the PA intensity, it is possible to distinguish between the two types of tissue. A commercial ultrasound system with a 128-element 5-14 MHz linear array transducer and a tunable ND:YAG laser were used to produce PA images. Ex-vivo bovine fat and porcine liver tissues were pre-cooled to below 10°C then warmed to 22°C over ~1 hour period. A thermocouple monitored the temperature rise while PA images were acquired at 0.5°C intervals. The averaged intensity of the illuminated tissue region at each temperature interval was plotted and linearly fitted. Liver samples showed a mean increase of 0.7%/°C, whereas bovine fat had a mean decrease of 1.5%/°C. By dividing each PA frame into smaller regions of interest (ROIs) and performing linear fitting over the subsequent frames, we obtained the slope values of each ROI. Our results showed that such mapping of the slope values can be used to differentiate fatty and non-fatty tissue types. For future clinical implementations, the temperature change can be also induced potentially by the laser heating when continuously acquiring PA frames over a period of time (e.g. 1 minute at 10 Hz repetition rate).

8943-184, Session PMon

Highly-sensitive optical microresonator sensor array for deep-tissue photoacoustic imaging

Jing Li, Edward Z Zhang, Paul C. Beard, Univ. College London (United Kingdom)

Currently, most photoacoustic scanners employ piezoelectric detectors. However, piezoelectric detectors have limitations such as restricting the delivery of the excitation laser beam and reduced sensitivity with decreasing element size. Planar Fabry Perot ultrasound sensing etalons can overcome these limitations and have proved to be extremely effective for superficial (<1cm) imaging applications. To achieve the acoustically small element sizes (~10's of microns) required for tomographic imaging, the etalon is typically illuminated with a focused laser beam. However, this has the disadvantage that beam walk-off due to the divergence of the beam fundamentally limits the etalon finesse and thus sensitivity – in essence, the problem is one of insufficient optical confinement. This has been addressed by using a novel plano-convex microresonator design, the geometry of which matches the wavefront of the interrogation laser beam. Due to the much stronger optical confinement provided by this approach, a significantly higher finesse yielding a sensitivity exceeding that of even the most sensitive piezoelectric detectors is theoretically achievable. To demonstrate the concept, an array of polymer microresonators has been fabricated using a novel inkjet printing approach to produce the cavity and physical vapour deposition to form the dielectric mirrors. These devices provide a noise equivalent pressure of 0.05kPa, an acoustic bandwidth of 10MHz and an effective element size of 100µm. This new approach offers the prospect of bringing the advantages of optical ultrasound detection to clinical breast imaging and other deep tissue photoacoustic applications previously the preserve of piezoelectric based detection schemes.

8943-185, Session PMon

Shear wave elastography at the micron scale using ultrafast full field OCT

Amir Nahas, Mickael Tanter, A. Claude Boccara, Institut Langevin (France)

Organ structures, tissues and cells are characterized by their intrinsic optical and mechanical properties. In this study we present a method that couples an ultrasound system and a fast FF-OCT in order to add the elastographic contrast to the FF-OCT images. To the best of our knowledge this is the first combination of a Full Field OCT setup with an ultrafast camera for real time 2D tracking of shear wave propagation. This method is a quantitative method based on shear wave propagation imaging. The ultrasound system is used to generate shear wave inside the sample and by using a very fast FF-OCT system (up to 30 kHz) we record the shear wave propagation at the scale given by the FF-OCT system (1 micrometer). As the local shear wave speed is directly related the local shear modulus, from the movie of the propagation we deduce a quantitative local stiffness. The signal-to-noise ratio of the fast FF-OCT system is high enough to work on various phantoms of different stiffness and with biological ex vivo tissue. Whereas the ultrasound scanner was able to track shear waves for ultrasonic radiation force "pushes" generating displacements of several micrometers, FF-OCT was able to detect and track shear waves for much smaller acoustic intensities (more than 100 fold).

8943-186, Session PMon

Imaging of blood vessels with CCD-camera based three-dimensional photoacoustic tomography

Robert Nuster, Karl-Franzens-Univ. Graz (Austria); Paul Slezak, Ludwig Boltzmann Institut (Austria); Günther Paltauf, Karl-Franzens-Univ. Graz (Austria)

Commonly real-time photoacoustic (PA) tomography setups are composed of an array of piezoelectric detectors and a multichannel data-acquisition system. Thereby it is possible to record time resolved signals simultaneously at several detector positions. An alternative approach is to use an optical phase contrast full field detection setup in combination with a CCD-camera to record acoustic fields instead of time resolved signals. This allows obtaining two-dimensional photoacoustic imaging in real-time, yielding one projection of the initial pressure distribution per excitation laser pulse.

The procedure for 3D-imaging is a three step process. First of all, with a defined delay time relating to the excitation laser pulse, 200 projection images of the 3D-wave pattern are recorded over an angular range of 180°. In the second step, projection images of the initial pressure distribution are acquired by back propagating the observed preprocessed wave pattern images in frequency space. In the final step, the inverse Radon transform is applied to the obtained projection dataset to reconstruct the initial three dimensional pressure distribution. Using a pulsed laser with 10 Hz repetition rate for PA excitation a three dimensional image can be obtained in less than 1 min. The sensitivity and resolution of the optical phase contrast detection system were estimated experimentally with 5 kPa mm without averaging and 66µm, respectively.

First experiments on a freshly excised rat muscle sample show the applicability of this technique for blood vessel imaging.

8943-187, Session PMon

Localized fluorescence excitation in opaque media by time-reversed ultrasonically encoded (TRUE) optical focusing

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To focus light beyond one transport mean free path, time-reversed ultrasonically encoded (TRUE) optical focusing has previously been implemented by both analog and digital devices. By allowing wavefront recording with finer resolution and larger aperture, the analog scheme, which uses photorefractive materials as the phase-conjugate mirror, generates a more complete set of time-reversed optical modes than the digital scheme. Here, we report the direct visualization of localized fluorescence excitation inside a turbid medium by photorefractive time reversal. Further, we imaged fluorescent targets embedded in a turbid phantom whose thickness was four transport mean free paths.

8943-56, Session 9

Noninvasive measurement of internal jugular venous oxygen saturation by photoacoustic imaging

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The metabolic rate and oxygen consumption of the brain is reflected in jugular venous oxygen saturation. In many clinical conditions, such as head trauma, stroke and low cardiac output states, the brain is at risk for hypoxic-ischemic injury. The current gold standard for monitoring brain oxygenation is invasive and requires jugular vein catheterization under fluoroscopic guidance, and therefore is rarely used. Photoacoustic measurement of oxygen extraction fraction and ultrasonic measurement of volumetric flow rate, can be used to estimate oxygen consumption of the brain in real-time. Such a noninvasive method will help earlier detection and prevention of impending hypoxic brain injury. A wavelength-tunable dye laser pumped by a Nd:YAG laser delivers light through an optical fiber bundle, and a modified commercial ultrasound imaging system (Philips iU22) detects both the pulse-echo ultrasound (US) and photoacoustic (PA) signals. A custom-built multichannel data acquisition system renders co-registered ultrasound and photoacoustic images at 5 frames per second. After localizing the jugular vein in healthy volunteers, dual-wavelength PA images were used to calculate the blood hemoglobin oxygen saturation from the internal jugular vein in vivo. The preliminary results raise confidence that this emerging technology can be used clinically for accurate, noninvasive measurement of cerebral oxygenation.

8943-57, Session 9

Three-dimensional tracking of lesion profile during laser surgery based on shock wave detection

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Lack of sensory feedback during laser surgery prevents surgeons from keeping track of the exact lesion profile and cutting depth. As a result, duration and complexity of the treatments are significantly increased.

In this study we propose a new method for enabling three-dimensional tracking of the exact lesion profile, based on detection of shock waves emanating from the ablated tissue and subsequent reconstruction of the incision location using time-of-flight data obtained from multiple acoustic detectors. Ablation was performed in fresh bovine tissue samples using a Q-switched Nd-YAG laser, delivering 8 ns duration 150mJ pulses at a wavelength of 1064nm and repetition rate of 5Hz. The beam was focused by a 50mm lens on the tissue surface, which resulted in a deep cut of up to 9mm depth. The generated shock waves were detected using a spherical matrix ultrasonic array. The exact cutting profile was subsequently rendered by reconstructing the origin of shockwaves detected during the entire procedure. Different combinations of the detector positions were considered with respect to the resulting reconstruction quality.

It was observed that, by utilizing at least 12 detection elements, the lesion profile could be characterized with high accuracy in all three dimensions, which was confirmed by histological evaluations. The proposed method holds promise for delivering highly precise and accurate real-time feedback during laser surgeries.

8943-58, Session 9

Photoacoustic imaging of mesenchymal stem cells in living mice via silica-coated gold nanorods

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Silica-coated gold nanorods (SiGNRs) were created with standard methods and loaded into mesenchymal stem cells (MSCs) without transfection agents. There was no significant ($p<0.05$) toxicity or changes to cell proliferation after incubating MSCs with 0.05 nM SiGNRs for 3 hours. A panel of cytokines should only minor upregulation of inflammatory markers including interleukin-6. We used electron microscopy to illustrate vacuole-bound SiGNRs inside the cells. This cell staining increased photoacoustic signal 175% relative to MSCs without contrast agent—the silica coat itself increased signal 55% relative to uncoated GNRs. Using inductively coupled plasma spectroscopy, we found that there were 100,000 SiGNRs per MSC. This value was 5-fold higher than a MSC population stained with GNRs in the absence of silica coat. After labeling, cells were washed and injected into murine muscle tissue to simulate a muscular dystrophy patient. Mice (N=5) treated with these SiGNR-labeled MSCs exhibited no adverse events and implants up to 5 mm deep were easily visualized. The in vivo detection limit was 90,000 cells in a 100 uL bolus in mouse thigh muscle ($p<0.05$). Here, the B-mode signal is useful for orienting the treatment area and visualizing the delivery catheter while the photoacoustic mode offers cell-specific content. The photoacoustic signal was validated with histology and a long-term fluorescent tracking dye after MSC transplant.

8943-59, Session 9

Characterization and treatment monitoring of inflammatory arthritis by photoacoustic imaging: a study on adjuvant-induced arthritis rat model

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In this study, we have successfully validated the capability of photoacoustic imaging (PAI) in evaluating angiogenesis in articular tissues in vivo for the purpose of diagnosing and monitoring the treatment of inflammatory arthritis. This study was conducted on a well-established adjuvant-induced arthritis (AIA) rat model which is a rodent model similar clinically and pathologically to human rheumatoid arthritis. To quantify the photoacoustic signal enhancement in the arthritic joint as a result of angiogenesis, the ankle joints of the rat were imaged at 532 nm. PAI of a joint was achieved by using a commercial ultrasound (US) unit without impacting its original imaging functions. This arrangement enables the same object to be scanned in both US and PAI modes using the same receiving probe at the same viewing angle, which facilitates very convenient off-line image co-registration. Since the joint structure can be clearly seen in the US image which is in real time, US was conducted before PAI to find the best view in visualizing articular tissues. US images of joints also help in interpretation and analysis of PAI outcomes. In order to validate the outcome from the PAI technique, we also imaged the same samples with positron emission tomography (PET) which, although involving ionizing radiation, has proved excellent sensitivity for inflammation and, therefore, was employed as a gold standard. The histological examination and the recording of clinical score of the imaged joints were also conducted, to verify angiogenesis and confirm arthritis in the joints.

8943-60, Session 9

Can photoacoustic imaging be used to differentiate photodynamic therapy responders from non-responders?

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Photodynamic therapy (PDT) is a clinically relevant treatment modality

that is used for various oncologic and dermatologic malignancies. PDT induces tissue cytotoxicity via excitation of a photo-activatable drug (photosensitizer) with a drug-specific wavelength of light. Most photosensitizers in their triplet-excited state generate cytotoxic species by reacting with the ground state oxygen that is available in the surrounding environment. Hence the PDT dose at the treatment site is determined by the following three factors: photosensitizer (PS) concentration, oxygenation status and delivered light dose.

Given the inter- and intra-subject variability in the uptake of the photosensitizer and the distribution of oxygen in the tumor, understanding the interplay between these dose parameters could aid in differentiating responders or non-responders to PDT. In this study, photoacoustic imaging with the Vevo LAZR system was used to monitor PS uptake and oxygenation changes to PDT in a subcutaneous tumor murine model. To validate if photoacoustic imaging can distinguish PDT responders from non-responders, we correlated the treatment response (reduction in tumor volume monitored for over 30 days) in these mice with information from photoacoustic images. We further compared the predictive capability of photoacoustic imaging with the more predominantly used fluorescence imaging in estimating photosensitizer consumption during PDT.

8943-61, Session 9

Preclinical evaluation of tumour response to vascular targeted therapy using photoacoustic imaging

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Vascular therapy in oncology uses the differences between normal blood vessels and abnormal tumour neo-angiogenesis to selectively target cancer. For optimal treatment efficacy, and translation of novel compounds, the response of the tumour vasculature needs to be assessed. Photoacoustic tomography (PAT) is capable of this as it provides highly spatially resolved 3D images of vascular networks in biological tissue to a depth of just under 1cm. In preclinical models of cancer this is sufficient to encompass entire subcutaneous tumours, and can therefore be used to evaluate pharmacological intervention directed at the vasculature. In this study the vascular disrupting agent OXi4503 was used to treat subcutaneous tumour mouse models of two human colorectal carcinoma tumour types (SW1222, LS174T) at a range of concentrations (40mg/kg, 10mg/kg, 1mg/kg and sham dose control). The characteristic destruction of tumour vasculature caused by OXi4503 was observed by PAT and confirmed ex vivo via histology. Differences observed between the two tumour types assessed demonstrate the importance of tumour microenvironment and pathophysiology on response to therapy. Differential response to different doses of OXi4503 was observed, with outward tumour growth only seen once entire tumour viability had been re-established; this demonstrates the potential of PAT to act as a biomarker of response for the translation of novel anti-vascular compounds and also within the clinic. This study shows clearly that PAT can accurately assess the time course of drug action and relapse of pharmacodynamic effect in preclinical models of cancer and the important translational prospects for vascular targeted tumour therapies.

8943-62, Session 9

Wavelength modulated differential photoacoustic spectroscopy (WM-DPAS) for ultrasensitive quantitative hemoglobin concentration and oxygenation monitoring in soft tissues

Sung soo (Sean) Choi, Bahman Lashkari, Xinxin Guo, Andreas Mandelis, Univ. of Toronto (Canada)

In the field of medical diagnostics, biomedical photoacoustic (PA) imaging is a non-invasive hybrid optical-ultrasonic imaging modality. Due to the unique hybrid capability of optical and acoustic imaging, PA imaging has risen to the frontiers of medical diagnostic procedures such as human breast cancer detection. While conventional PA imaging has been mainly carried out by a high-power pulsed laser, an alternative technology, Frequency Domain Biophotoacoustic Radar (FD-PAR) is under intensive development. It utilizes a continuous wave optical source with the laser intensity modulated by a frequency-swept (chirped) waveform for acoustic wave generation. The small amplitude of the generated acoustic wave is significantly compensated by increased signal-to-noise ratio by several orders of magnitude using matched-filter correlation processing in a way similar to radar systems.

The current study introduces a novel FD-PAR modality for ultra-sensitive characterization of functional information for breast cancer imaging. Wavelength-modulated differential PA spectroscopy (WM-DPAS) detection has been developed to address angiogenesis and hypoxia monitoring, two well-known benchmarks of breast tumor formation. Based on WM-DPAS theory, this modality efficiently suppresses background absorptions and is expected to detect very small changes in total hemoglobin concentration and oxygenation levels, therefore identifying pre-malignant tumors before they are anatomically apparent. Preliminary single-ended laser experimental results were compared to a developed theoretical formalism. More extended differential measurements using two wavelength-modulated lasers (~680nm and ~800nm) are being investigated and will be reported for the sensitive assessment of the total hemoglobin concentration and oxygenation level, thus indicating the health state of tissues in the biological tissue.

8943-63, Session 10

High-speed intravascular photoacoustic catheter for atherosclerotic artery imaging

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Photoacoustic imaging using the intrinsic contrast from harmonic vibration of C-H bonds allows selective mapping of lipids deposition inside the artery wall. The current developed intravascular photoacoustic endoscopes employ 10 Hz repetition excitation at 1730 nm from a Nd:YAG pumped OPO system, which is 2 orders of magnitude slower than the speed for in vivo requirement. Towards the goal of diagnosis atherosclerosis in clinical setting, we herein demonstrate a high-speed photoacoustic catheter based on a Raman laser with 1 KHz repetition rate for intravascular lipid visualization. In our study, 1730 nm excitation from a 1 KHz Raman laser was used to excite the first overtone vibration of C-H bonds. By scanning the probe with a combination of rotary and linear stages, three dimensional imaging of atherosclerotic plaques was performed. Our study enables the deployment of the photoacoustic catheter towards in vivo applications.

8943-64, Session 10

Structured-illumination photoacoustic Doppler flowmetry of axial flow in homogeneous scattering media

Ruiying Zhang, Junjie Yao, Konstantin I. Maslov, Lihong V. Wang, Washington Univ. in St. Louis (United States)

We propose a new flow speed measurement method based on the photoacoustic Doppler effect in a homogeneous medium. Unlike the back-scattering based Doppler methods, the new method does not require heterogeneity of the flowing medium. As long as the medium is optically absorptive at a certain wavelength, its flow speed can be measured based on the photoacoustic Doppler effect. A spatially modulated pulsed laser was used to temporally modulate the received PA signals. The modulation frequency of the received PA signals is determined by the acoustic transit time between the neighboring pitches of the spatial modulation. Therefore, the flow of the medium changes the acoustic transit time and thus induces a frequency shift in the received PA signals. First, four calculation methods were explored. Then based on these four methods, a red-ink phantom flowing in a tube immersed both in water and scattering media was measured. We believe this new method can be used for numerous biomedical studies, especially where high particle concentrations are required.

8943-65, Session 10

Two-photon absorption-induced photoacoustic and luminescence imaging employing a femtosecond laser

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Two-photon photoacoustic microscopy (TP-PAM) can be considered as the photoacoustic analogue of two-photon laser scanning fluorescence microscopy. In both cases a highly focused pulsed laser beam is scanned over a sample. In two-photon laser scanning fluorescence microscopy the fluorescence of the sample is recorded, while in TP-PAI ultrasonic signals caused by the rapid temperature rise due to light absorption are measured. We expect that the advantages of two-photon photoacoustic microscopy are similar to those of two-photon laser scanning fluorescence microscopy, e.g. deeper tissue penetration, and better axial resolution.

In this work we demonstrate multimodal photoacoustic and luminescence microscopy via two-photon absorption, and show the possibility to image objects with localized dyes. The excitation is performed via a femtosecond laser with a wavelength of 800nm. Simultaneously to the measurement of the photoacoustic signals we detect the luminescence intensity and its spectrum. In order to check if our measurement and evaluation method gives correct results we determine the photoacoustic and luminescence response of samples exhibiting single- and two-photon absorption. For single-photon absorption the photoacoustic signal is linear proportional to the excitation intensity and for two-photon absorption it is quadratic. Our results match the theoretical expectations.

Finally, we simultaneously record photoacoustic and luminescence images, e.g. of dyed microspheres, and show that the photoacoustic and luminescence images match. The recorded luminescence spectra do not give any hint for second harmonic generation, optical breakdown or other non-linear effects that could cause single-photon absorption. Therefore we conclude that the photoacoustic signals are due to two-photon absorption.

8943-66, Session 10

Photoacoustic correlation spectroscopy for calibration-free absolute quantification of particle concentration

Yong Zhou, Junjie Yao, Konstantin I. Maslov, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Over the past few years, photoacoustic microscopy (PAM) has been proven to be capable of structural, functional, molecular, and metabolic imaging. Currently, laser fluence calibration is typically required for quantitative measurement of particle concentration in PAM. In this paper, we present another quantitative approach, one that measures absolute absorber concentrations by photoacoustic correlation spectroscopy. The proposed method is based on the fact that Brownian motion induces particle count fluctuation in the detection volume. If the count of particles in the detection volume is assumed to follow a Poisson distribution, its expected value can be calculated by the photoacoustic signal variance. To obtain a fluence-independent detection, the particle count in the detection volume needs to be small enough that the PA signal fluctuation due to the particle Brownian motion is dominant over other fluctuating sources, such as laser intensity fluctuation, electronic thermal noise, and photon shot noise. In this paper, first, a theoretical model was derived for photoacoustic signals. Then, we applied our method to quantitative measurement of different concentrations of various particles, including red blood cells. We show that our method can measure as few as three RBCs in the detection volume. The experimental results agreed well with the predictions from the theoretical model, suggesting that our method potentially can be used for noninvasive measurement of absolute particle concentrations in deep tissue imaging, without fluence calibration.

8943-67, Session 10

Multimodality photoacoustic and Raman imaging of magnetically-trapped tumor cells

Wei Shi, Peng Shao, Robert J. Paproski, Univ. of Alberta (Canada); Alexander Forbrich, University of Alberta (Canada); Roger J. Zemp, Univ. of Alberta (Canada)

Photoacoustic microscopy (PAM) is an emerging imaging technology for visualizing optically-absorbing superficial structures in vivo with high resolution and high imaging contrast. However, the differentiation of signals is nontrivial hence the specificity of this technology can be poor. Raman imaging technology for imaging surface-enhanced Raman scattering nanoparticles (NPs) is previously demonstrated to have extremely high specificity and multiplexing capabilities due to narrow spectral features of the Raman spectrum. Here we present a new integrated multimodality imaging system combing PAM and Raman imaging technology for structural and functional imaging of microvasculature and detecting & identifying magnetically-trapped circulating tumor cells (CTCs) simultaneously.

In our system, SERS NPs are composed of a 60 nm-diameter Au core adsorbed with a Raman active molecular monolayer and encapsulated with a 30 nm diameter silica shell. Mixtures of folate conjugated SERS NPs& fluorescent magnetic NPs are used for targeting tumor cells with folate receptors. Incorporating a custom Raman imaging spectrometer with an EMCCD camera and a 785 nm excitation laser and an objective lens (shared with PAM sub-system), the lowest concentration of SERS NPs can be detected is 6pM. Classic least-squares method is used for de-mixing. A highly linear relationship between estimated concentrations and actual concentrations of SERS NPs is presented with R2=0.99. Because of high multiplexing capabilities, SERS nanoparticles offer outstanding potential for high-specificity detection of circulating tumor cells.

By using a diode-pumped nanosecond-pulsed Ytterbium-doped 532-nm fiber laser with pulse-repetition rate up to 600 kHz combined with fast-scanning mirrors, our PAM sub-system is capable of near real-

time C-scan and 3D photoacoustic imaging with ~6-μm lateral spatial resolution.

In vitro research on cells flowing inside tubing and in vivo studies on rat ear demonstrated that our system is able to provide superimposed imaging of structural imaging with multiplex detection of magnetically-trapped CTCs.

The system can be used for clinic applications, helping visualize angiogenesis and identifying the CTCs in the circulatory system, as well as aiding in in vitro studies of CTCs by drawing out appropriate amount of CTCs.

8943-68, Session 10

Label-free mouse brain imaging by high-speed functional photoacoustic microscopy

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With the development of genetically altered mouse models, various human neurological diseases can be reproduced in mice. Mouse brain imaging has provided valuable information for fundamental neuroscience and translatable insights for clinical neurology. Existing small-animal imaging techniques, such as functional magnetic resonance imaging (fMRI), X-ray computed tomography (CT), single-photon emission computed tomography (SPECT), and positron emission tomography (PET), have played important roles in mouse brain imaging. However, these techniques lack either the spatial resolution needed for cortical microvasculature study or the imaging speed required for dynamic brain study. Two-photon microscopy has been widely used for small-animal brain imaging with high spatial resolution and imaging speed, but it requires exogenous fluorescent contrast agents and the field of view is limited to a few hundred micrometers. Here, we introduce high speed functional photoacoustic microscopy (PAM) that can be utilized for mouse brain imaging where observations of time-sensitive dynamic changes are critical. PAM has achieved a capillary-level resolution of ~3 μm and a cross-sectional imaging speed of ~400 Hz. To demonstrate the multiparametric imaging capability of PAM, we studied microhemodynamics of the mouse brain cortex in an intact skull in response to various physiological and pathological challenges. The results collectively suggest that PAM could be a promising tool for understanding various neurophysiological phenomena, while complementing conventional imaging modalities.

8943-69, Session 10

Intraoperative surgical photoacoustic microscopy using augmented reality

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We have developed an intraoperative surgical photoacoustic microscopy (IS-PAM) system by integrating an optical resolution photoacoustic microscopy (OR-PAM) and conventional surgical microscope. Based on the common optical path in the OR-PAM and microscope system, we can acquire the PAM and microscope images at the same time. Furthermore, by utilizing a mini-sized beam projector, 2D PAM images are back-projected onto the microscope view plane as augmented reality. Thus, both the conventional microscopic and 2D cross-sectional PAM images are displayed on the plane though an ocular lens of the microscope. In our method, additional image display tool is not required to show the PAM image. Therefore, it potentially offers significant convenience to surgeons without movement of their sights during surgeries. In order to demonstrate the performance of our IS-PAM system, first, we successfully monitored needle intervention in phantoms. Moreover, we successfully guided needle insertion into mice skins in vivo by visualizing surrounding blood vessels from the PAM images and the magnified skin surfaces from the conventional microscopic images simultaneously.

8943-70, Session 11

Alternative to the sentinel lymph node biopsy: ultrasound-guided spectroscopic photoacoustic imaging of molecularly-activatable plasmonic nanosensors

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The majority of cancer deaths result from metastases, and the development of microscopic regional lymph node metastases is often a harbinger of clinically significant nodal or distant metastatic disease. However, due to low sensitivity and specificity of current imaging methods to detect early regional spread of the tumor, an invasive surgical procedure – sentinel lymph node (SLN) biopsy – is currently used to identify metastatic cancer cells. We have developed a non-invasive approach to image micrometastases in the lymphatics using ultrasound-guided spectroscopic photoacoustic imaging of bioconjugated spherical gold nanoparticles. Using a metastatic murine model of squamous cell carcinoma of the oral cavity, we show that gold nanoparticles targeted to the epidermal growth factor receptor change optical properties upon interaction with epidermal growth factor receptor overexpressing metastatic cancer cells and, therefore, act as molecularly-activated plasmonic nanosensors (MAPS). The MAPS selectively interact with LN micrometastases resulting in a significant increase in sPA signal over controls and 85.7% sensitivity and 87.5% specificity. Furthermore, we were able to detect metastatic foci as small as 50 μm using our method. We envision that the eventual clinical translation of this technology could significantly improve the ability of physicians to stage cancer patients. By detecting micrometastases in real-time and noninvasively using MAPS, the SLN biopsy, and its associated morbidity, could be avoided.

8943-71, Session 11

Methylene blue conjugated microbubbles (MB2) as a dual modal contrast agent for photoacoustic and ultrasound imaging

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We have demonstrated microbubbles (a commonly used ultrasound contrast agent) in a solution of methylene blue (a commonly used

photoacoustic dye), in a novel "MB2" solution for dual modality contrast. To investigate the dual modal imaging capability of MB2, we photoacoustically and ultrasonically imaged aqueous solutions of MB2 by varying the concentration of either microbubbles or methylene blue. Interestingly, as the microbubble concentration increased (with the methylene blue concentration constant), photoacoustic signal was greatly attenuated in the MB2 solution. Conversely, when methylene blue concentration increased (with microbubble concentration constant), no interference was observed. To further confirm our findings, we studied the switching of photoacousitc and ultrasound signals using sonication. We compared the photoacoustic and ultrasound signals of the MB2 solution before and after sonication. The photoacoustic amplitude increased 2.5 times. Conversely, the ultrasound signals were initially strong, but decreased 2.5 times following sonication. The photoacoustic signals consequently increased due to greater optical absorption in the solutions for increasing the concentration of methylene blue. However, the ultrasound intensities remain same because of the fixed bubble concentration. Moreover, we used a clinically modified ultrasound imaging scanner to disrupt the microbubbles in MB2 and recover the PA signals. Clinical ultrasound imaging scanner for generating high powered ultrasound burst the microbubbles and the drastically recovered photoacoustic signal enhancement was to ~817 times. Because both methylene blue and microbubbles are widely used in current clinical practices, our novel dual modal agent, MB2, is highly translational.

8943-72, Session 11

Photo-thermal stimuli responsive theranostic agents for signal enhanced photoacoustic imaging

Yun-Sheng Chen, Soon Joon Yoon, Mary Dockery, Wolfgang Frey, Stanislav Y. Emelianov, The Univ. of Texas at Austin (United States)

Photoacoustic imaging is an attractive imaging modality for translational molecular imaging and for guiding photothermal therapy noninvasively. In these applications, nanometer size imaging theranostic agents are usually essential, and play an important role by molecular specifically targeting to the imaging targets and enhancing the photoacoustic responses, thus, improving the imaging contrast and sensitivity. In this study, we designed and prepared new photoacoustic theranostic agents using thermal stimuli sensitive polymer/gold nanorod nanocomposates. The developed agents, while inherit superior optical properties from gold nanorods such as strong and tunable optical absorption, show enhanced photoacoustic signal attributed to the aggregation of gold nanorods in polymer matrix. Because of the restorable thermal response, the thermal stimuli polymer matrix allows us to further control the aggregation status and thus to manipulate the photoacoustic response through photothermal heating dynamically. Using this photo-switchable nature of the photoacoustic response, we demonstrate a positive photoacoustic dynamic contrast mechanism, which leads to further enhancement of the imaging contrast, especially appealing in photothermal cancer imaging and therapy.

8943-73, Session 11

Optimizing properties of photoacoustic nanodroplets for photoacoustic and ultrasound image contrast

Alexander Hannah, Donald VanderLaan, Stanislav Y. Emelianov, The Univ. of Texas at Austin (United States)

Photoacoustic signal is usually based on thermoelastic expansion of optical absorbers, which results in only modest contrast in a biological medium. Recently, photoacoustic nanodroplets have been developed—perfluorocarbon droplets with encapsulated photoabsorbers—which undergo a liquid-to-gas transition upon triggering by pulsed laser irradiation. This vaporization leads to a strong acoustic emission, which enhances photoacoustic image contrast to a greater degree than dyes and plasmonic particles do. The vaporized bubbles also provide ultrasound contrast, resulting in a dynamic imaging construct. Various formulations of nanodroplets have been developed, and their features can be adjusted for optimal performance. We have investigated properties that are applicable to an in vivo setting, including droplet stability, absorption wavelength, safe clinical translation, and vaporization efficiency. We have developed droplets with a variety of photoabsorbers, including dyes which are biocompatible, as well as dyes and plasmonic nanorods which absorb light at 1064 nm, improving image contrast. In addition, droplet stability, sensitivity, and vaporization efficiency can be controlled by mixing different types of perfluorocarbons of various boiling points, thus altering circulation time, fluence threshold for vaporization, and imaging depth. We also investigate the mechanism of vaporization by observing droplet behavior under various optical and acoustic parameters. These photoacoustic nanodroplets provide high contrast for medical imaging of tumors, and may also encapsulate therapeutics for image guided, triggered release of drugs and subsequent monitoring of disease progression.

8943-74, Session 11

Lipid-stabilized porphyrin-containing perfluorobutane nanodroplets: submicron ultrasound and photoacoustic contrast imaging agents

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Liposomes containing porphyrins (natural biological molecules which strongly absorb light) have been described as multimodal photonic contrast agents capable of enhancing photothermal tumor ablation (Lovell et al. Nature Materials 2011). Microbubbles containing porphyrins have previously been reported which can also provide ultrasound contrast although these particles were 2-4 microns in size and would thus remain in the vasculature in vivo. In order to create an ultrasound/photoacoustic contrast agent capable of accumulating in tumors, we have created submicron porphyrin-containing perfluorobutane nanodroplets. A Nanosight LM10-HS system confirmed the submicron size of the porphyrin-nanodroplets. Heating of porphyrin-nanodroplets by ultrasound or laser irradiation was capable of phase-changing the nanodroplets into microbubbles as confirmed by microscopy. Porphyrin-nanodroplets were also verified as photoacoustic contrast agents by phantom imaging studies. Preliminary in vivo data suggests that our nanodroplets can significantly accumulate in xenograft Hep3 tumors in chicken embryos, suggesting that our porphyrin-nanodroplets can provide contrast for both ultrasound and photoacoustic imaging of tumors.

8943-75, Session 11

Nonlinear acoustic enhancement in integrated ultrasound/photoacoustic imaging with wideband absorptive nanoemulsion beads

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A new type of contrast agent, a nanoemulsion with a perfluorohexane core and highly absorptive gold nanospheres (GNSs) assembled on the surface, is presented to improve the specificity of photoacoustic (PA) molecular imaging in differentiating targeted cells or aberrant regions from heterogeneous background signals. Compared to distributed GNSs, clustered GNSs at the emulsion oil-water interface produce a red-shifted and broadened absorption spectrum, exhibiting fairly high absorption in the near-infrared region commonly used for deep tissue imaging. Above a certain laser irradiation fluence threshold, a phase transition creating microbubble cavitation in the emulsion core leads to very strong PA signals compared with conventional thermal-expansion-induced PA signals. These signals are also strongly non-linear, as verified by a differential scheme using recorded PA images at different laser fluences. Assuming a linear relation between laser fluence and the PA signal amplitude, differential processing results in nearly perfect suppression of linear sources, but retains a significant residue for the non-linear nanoemulsion with more than 20 dB enhancement. This result demonstrates that contrast specificity can be improved using the nanoemulsion as a targeting agent in PA molecular imaging by suppressing all background signals related to a linear PA response. Furthermore, combined with a system providing simultaneous laser/ultrasound excitation, cavitation-generated bubbles have the potential to be a highly specific contrast agent for ultrasound molecular imaging and harmonic imaging, as well as a targeted therapeutic agent for high intensity focused ultrasound (HIFU) applications.

8943-76, Session 11

Poly-N-isopropylacrylamide (PNIPAM) nanoclusters as photoacoustic imaging contrast agents

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Gold nanoparticles have attracted tremendous interest in photoacoustic imaging due to their strong optical absorption in the visible and near-infrared regions. In this paper, metal-hydrogel nanocomposites consisting of gold nanospheres encapsulated within thermo-responsive poly-N-isopropylacrylamide (PNIPAM) nanogel carriers as a contrast agent for photoacoustic imaging were synthesized and investigated. Using a UV-Vis spectrometer, the extinction spectra of PNIPAM nanoclusters were measured at various temperatures. As temperature increases, the plasmon resonance peak broadens due to the smaller interparticle distance. The overall size of the PNIPAM nanoclusters was measured by Dynamic Light Scattering as a function of temperature. The PNIPAM gold nanoclusters were also used to verify the photoacoustic signal enhancement from clustering of plasmonic nanoparticles. Agar based tissue mimicking phantoms consisting of inclusions of thermo-sensitive PNIPAM nanoclusters were used to control the distance between primary nanoparticles. To change the interparticle distance of PNIPAM gold nanoclusters, the temperature of the phantom was set to room temperature and then increased stepwise. Photoacoustic imaging results prove that the PNIPAM gold nanoclusters have the ability to enhance photoacoustic signal. These results indicate that the PNIPAM nanoclusters of gold nanoparticles can be used as efficient contrast agents in photoacoustic imaging. In addition, the thermo-stimulus nature of PNIPAM nangel carriers will allow for release of drugs or other therapeutic agents in future applications.

8943-77, Session 12

Patterned interrogation scheme for compressed sensing photoacoustic imaging using a Fabry Perot planar sensor

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Photoacoustic tomography (PAT) has become a powerful tool for biomedical imaging, particularly pre-clinical small animal imaging. Several different measurement systems have been demonstrated, and optically addressed Fabry-Perot interferometer (FPI) sensor arrays have been shown to provide exquisite images when a planar array geometry is suitable. However, in its current incarnation the measurements must be made at each point sequentially, so these devices therefore suffer from slow data acquisition time. An alternative to this point-by-point interrogation scheme, is to interrogate the whole sensor with a series of independent patterns, so each measurement is the spatial integral of the product of the pattern and the acoustic field (as in the single-pixel Rice camera). For a suitably chosen set of patterns, such a scheme allows compressed sensing to be used. This enables the number of measurements to be reduced significantly, leading to much faster data acquisition, and the prospect of achieving 3D imaging at video rates.

An experimental implementation will be described, which employs a wide NIR tunable laser beam to interrogate the FPI sensor. The reflected beam is patterned and focused to a single photodiode by a digital micro-mirror device. To demonstrate the idea of patterned illumination and compressed sensing for ultrasound detection, both Hadamard and random binary patterns are utilised in the experiments. The results of detection acoustic signals from a 15MHz transducer and photoacoustic signals from phantom study will also be presented.

8943-78, Session 12

Near infrared optical coherence photoacoustic microscopy (NIR-OC-PAM)

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We built an optical coherence photoacoustic microscope (OC-PAM) using a single near infrared (NIR) pulsed broadband laser source. The light source has a center wavelength of 808 nm and a FWHM bandwidth of 70 nm. A free-space interferometer was used to construct the OCT image which detects the combined reflected light from the sample and the reference arms. In the meantime an ultrasonic needle transducer was used to detect the photoacoustic signals to accomplish photoacoustic imaging. The NIR-OC-PAM can provide simultaneous high resolution scattering and absorption contrasts of a sample.

We first tested the system on imaging a phantom made of Intralipid and ink to simulate the scattering and absorption properties of biological samples. The capability of the system to extract quantitative scattering and absorption information of biological samples was examined with the phantom. To test the capabilities of the system on ophthalmic application we imaged the retina of pigmented rats. The OCT images revealed the retinal structures with quality similar to conventional OCT while the PAM images revealed the distribution of absorbers in the retina like melanin in the retinal pigment epithelium. Generated from the same group of photons the OCT and PAM images are intrinsically registered. Since the absorption of hemoglobin is relatively weak in the NIR, images of the retinal blood vessels are not as good as that acquired with visible light photoacoustic ophthalmoscope (PAOM).

In conclusion, we believe the progress on NIR-OC-PAM will benefit not only ophthalmic imaging study but also the field of tissue engineering.

8943-79, Session 12

Two-wavelength identification of lipid in atherosclerotic plaques by intravascular photoacoustic imaging at 1.7 μm

Min Wu, Krista Jansen, Antonius F. W. van der Steen, Gijs van Soest, Erasmus MC (Netherlands)

Lipid contents in human atherosclerotic plaques are important markers to detect atherosclerotic lesions and disease states. Intravascular photoacoustic imaging (IVPA) is a promising new tool to detect lipids in atherosclerotic coronary lesions on the basis of the optical absorption contrast between different tissue types. Lipids are highly effective absorbers at wavelengths near 1720 nm due to the first overtone of the C-H bonds which are abundant in lipids. In this study, combined intravascular ultrasound (IVUS) and photoacoustic imaging (IVPA) system was used to further investigate the performance of lipid detection by IVPA imaging in the 1.7 μm spectral range. Ex vivo IVPA imaging on a vessel phantom and human coronary arteries was performed at several wavelengths between 1660 nm and 1760 nm. The observed spectra of atherosclerotic plaque lipids and peri-adventitial lipids were relatively similar, and requiring a careful wavelength selection to distinguish between them. By exploiting the relative difference between the IVPA signal strengths at 1718 nm and 1734 nm wavelengths, both plaque lipids and peri-adventitial lipids can be successfully detected and even distinguished. The threshold difference is determined based on the statistical analysis of hundreds of spectral data obtained from phantom measurements. Our study demonstrates that IVPA imaging can positively identify atherosclerotic plaque lipids with only two wavelengths, enabling fast data acquisition in vivo.

8943-80, Session 12

Vibrational photoacoustic tomography: chemical imaging beyond the ballistic regime

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Inherent molecular vibration offers a contrast mechanism for chemical imaging in a label free manner. In vibrational microscopy based on either infrared absorption or Raman scattering, the imaging depth is limited to the ballistic photon mean free path, which is a few hundred microns in a biological sample. Owing to much weaker acoustic scattering in tissues as compared to optical scattering, photoacoustic detection of harmonic molecular vibration has enabled significant improvement in imaging depth. In this paper, vibrational photoacoustic tomography is demonstrated with a homebuilt Raman laser generating greater than 100 mJ pulse energy at 1197 nm wavelength. This system was employed for the excitation of second overtone transition of C-H bonds. Vibrational photoacoustic signal from C-H rich polyethylene tube phantom placed under 3 cm thick chicken breast tissue was obtained with a signal to noise ratio of 2.5. Further, we recorded photoacoustic images of a polyethylene ring and fatty liver placed under 5 mm chicken tissue with excellent contrast. No phototoxicity to cells was seen at the laser energy used for tomography. This development opens potential applications of using label free vibrational techniques to image the deep tissue regime.

8943-81, Session 12

Feasibility of modulation-encoded TOBE CMUTs for single-shot 3D photoacoustic imaging

Ryan K. Chee, Roger J. Zemp, Univ. of Alberta (Canada)

We recently introduced top orthogonal bottom electrode (TOBE) capacitive micromachined ultrasound transducer (CMUT) 2D arrays and showed how dominant single element control could be achieved by biasing a column and transmitting or receiving across a row. A typical TOBE CMUT can acquire an entire column of data simultaneously. Thus using only N transmit/receive channels and N bias channels novel imaging schemes could be achieved without needing N2 channels for a NxN array. Previously proposed imaging schemes, however, required multiplexing to form images. In principle, if all elements of a 2D array could route signals, 3D photoacoustic imaging would be possible with a single laser shot. Unfortunately, fully-wired 2D arrays become difficult and cost-prohibitive as the size of the array becomes large or for small elements or catheter applications. We present a highly novel scheme for equivalently extracting signals from each element of a TOBE CMUT 2D array with only N receive channels. The method involves modulating columns using frequencies >100MHz, far above the resonant-frequency bands of the CMUTs. Each column is modulated at a distinct frequency. Photoacoustic signals inducing nonlinear capacitive oscillations in CMUT cells result in sidebands about the modulation bands. Signals are received across rows and digitized at GHz sampling frequencies. Photoacoustic signals from each element are recovered by beating the row signals against respective modulation frequencies and low-pass filtering. We demonstrate the feasibility of this approach by acquiring several frequency modulated data points from a single row simultaneously. We also detail the theoretical background behind this approach and we verify experimentally some key theoretical predictions such as the linear increase in signal in response to carrier amplitude, bias voltage, and applied signal amplitude. Signal amplitude vs. cross section data is also presented. Photoacoustic signals are recorded from a small 2D TOBE CMUT array. Larger effective arrays are synthesized by mechanical scanning to demonstrate proof of principle of high-quality images. Through this feasibility study, we demonstrate a promising new approach for greatly increasing the speed of photoacoustic imaging, and for complete and parallel addressing of dense 2D arrays without requiring complete wiring.

8943-82, Session 12

Photoacoustic imaging with TOBE CMUTs

Ryan K. Chee, Univ. of Alberta (Canada); Deepak Rishi, BITS Pilani (India); Alexander Sampaleanu, Roger J. Zemp, Univ. of Alberta (Canada)

Recently we introduced top orthogonal to bottom electrode (TOBE) capacitive micromachined ultrasound transducer (CMUT) 2D arrays. As the name suggests, top electrodes are routed in rows orthogonal to bottom electrode columns. We have shown that only cells at the intersection of a biased column and a transmitting or receiving row experience appreciable actuation or receive sensitivity. This demonstration of effective single element control is significant because an NxN array can be addressed using only N transmit-receive channels and N bias channels, rather than N2 electronic channels. In this paper, we present the first photoacoustic images taken from our TOBE CMUTs. We present a photoacoustic images of a phantoms in immersion using a synthetic aperture and digital beam-forming. We acquired the image using a combination of electronic scanning and mechanical scanning to demonstrate feasibility for acquisition with very large 2D arrays. The image acquired has resolution consistent with the theoretical predictions. Our TOBE arrays offers 3D imaging capabilities with N laser shots for an NxN array. Noise equivalent pressures were ~100mPa/ for each element of the TOBE. Sensitivities are even greater after focusing. TOBE CMUT arrays offer potential to scale 2D arrays to very large dimensions, opening up new possibilities for ultrasound and photoacoustic imaging.

8943-83, Session 12

Photoacoustic imaging with multi-frequency CMUT arrays

Ryan K. Chee, Univ. of Alberta (Canada); Deepak Rishi, BITS Pilani (India); Alexander Sampaleanu, Roger J. Zemp, Univ. of Alberta (Canada)

Multi-frequency transducers could enable ultra-wideband and multi-scale photoacoustic imaging. Such arrays would also have significant potential for image-guided therapies, wherein low-frequency cells could be used for high-intensity focused ultrasound and high-frequency cells for imaging. Unfortunately multi-frequency piezoelectric transducers are difficult to fabricate. CMUT (capacitive micromachined ultrasound transducer) arrays may prove a sensitive alternative. We demonstrate photoacoustic imaging using multi-frequency CMUT arrays. Our multi-frequency arrays consist of multiple 66 by 2 CMUT arrays with interlaced 82 micrometer and 36 micrometer devices, corresponding to center frequencies of 1.5MHz and 6.5MHz in immersion. Array element pitch is roughly 1 lambda for the 6.5MHz band and close to lambda/4 for the 1.5MHz band. Images are acquired from a hair and carbon fiber phantoms in immersion using a synthetic aperture and digital beam-forming. The results demonstrate the ability of our multi-frequency CMUT to achieve broadband photoacoustic images. Point-spread functions acquired with the 82 micrometer CMUT cells offer lower resolution but deeper penetration than those acquired with 36 micrometer devices. Ongoing work includes interfacing for fully parallel receive acquisition using an array ultrasound platform and extending these phantom studies to in vivo imaging applications.

8943-188, Session PTues

Dual-modality photoacoustic and ultrasound imaging system for noninvasive sentinel lymph node detection: preliminary clinical results

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Sentinel lymph node biopsy (SLNB) has emerged as an accurate, less invasive alternative to axillary lymph node dissection, and it has rapidly become the standard of care in patients with clinically node-negative breast cancer. The sentinel lymph node (SLN) hypothesis states that the pathological status of the axilla can be accurately predicted by determining the status of the first (i.e., sentinel) lymph nodes that drain from the primary tumor. Physicians use radio-labeled sulfur colloid and/or methylene blue dye to identify the SLN, which is most likely to contain metastatic cancer cells. However, the surgical procedure causes morbidity and associated expenses. To overcome these limitations, we developed a dual-modality photoacoustic and ultrasound imaging system to noninvasively detect SLN based on the accumulation of methylene blue dye. Ultimately, we aim to guide percutaneous needle biopsies and provide a minimally invasive method for axillary staging of breast cancer. The system consists of a tunable dye laser pumped by a Nd:YAG laser, a commercial ultrasound imaging system (Philips iU22), and a multichannel data acquisition system which displays co-registered photoacoustic and ultrasound images in real-time. The preliminary clinical results demonstrate that real-time photoacoustic imaging can provide sensitive and specific detection of methylene blue dye in vivo. While preliminary studies have shown that in vivo detection of SLNs by using co-registered photoacoustic and ultrasound imaging is feasible, further investigation is needed to demonstrate robust SLN detection.

8943-189, Session PTues

Realtime clinically-oriented array-based in vivo combined photoacoustic and power doppler imaging

Tyler J. Harrison, Dean Jeffery, Edward Wiebe, Univ. of Alberta (Canada); Roger J Zemp, Univ of Alberta (Canada)

Photoacoustic imaging has great potential for identifying vascular regions for clinical imaging. In addition to assessing angiogenesis in cancers, there are many other disease processes that result in increased vascularity that present novel targets for photoacoustic imaging. Doppler imaging can provide good localization of large vessels, but poor imaging of small or low flow speed vessels and is susceptible to motion artifacts. Photoacoustic imaging can provide visualization of small vessels, but due to the filtering effects of ultrasound transducers, only shows the edges of large vessels.

Thus, we have combined photoacoustic imaging with ultrasound power Doppler to provide contrast agent-free vascular imaging. We use a research-oriented ultrasound array system to provide interlaced ultrasound, doppler, and photoacoustic imaging. This system features realtime display of all three modalities with adjustable persistence, rejection, and compression. For ease of use in a clinical setting, display of each mode can be disabled. We verify the ability of this system to identify vessels with varying flow speeds using receiver operating characteristic curves, and find that as flow speed falls, photoacoustic imaging becomes a much better method for identifying blood vessels. We also present several in vivo images of the thyroid and several synovial joints to assess the practicality of this imaging for clinical applications.

8943-190, Session PTues

Source-receiver photoacoustic wave interferometry

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The representation theorems of the convolution type and the correlation type are used to obtain the superposition of the Green's function and its time reversal counterpart for the photoacoustic wave equation. Based on the reciprocity theorems, an interferometry relation providing the Green's function between sources and receivers is obtained. Three dimensional simulations are performed using finite element method to demonstrate the feasibility of virtual sources. Our results indicate that the virtual source method can be used for photoacoustics. The methodology given in this work shows that the measurement that would be observed at one of these detectors if there were a photoacoustic point source at the other one. It is important to note that no new data is created. In fact, virtual source method enables the use of data excluded in conventional imaging techniques. The virtual source approach can make it possible to replace noisy measurements with fictitious measurements. The implementation of virtual source method to photoacoustic imaging may provide some crucial advantages over the conventional photoacoustic methods. Passive imaging can also offer reduced data acquisition time since resolution is a crucial concern for any imaging modality. If the virtual sources are used, it is possible to reduce the number of real sources. This approach may be useful in small animal and human imaging studies.

8943-191, Session PTues

Near-infrared optical-resolution photoacoustic microscopy at 1046 nm wavelength

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The near-infrared (NIR) optical window is usually defined as the wavelength range where the penetration depth of light in biological tissue is maximized due to low optical absorption. Within the near-infrared optical window, photoacoustic imaging is currently limited to contrast-agents enhanced applications. Here we applied a 1046 nm wavelength, a typical wavelength in the near-infrared optical window, in optical-resolution photoacoustic microscopy (OR-PAM). A NIR-OR-PAM system with dual-wavelength illumination at 1046 nm and 570 nm was built. We compared the imaging depth and resolution at different depths at both wavelengths. We also compared in vivo images of a mouse ear and brain vascular network at both wavelengths and revealed several benefits of 1046 nm NIR-OR-PAM. First, 1046 nm NIR-OR-PAM can penetrate deeper than 570 nm OR-PAM, 3.2 mm compared with 2.3 mm. Second, 1046 nm NIR-OR-PAM has better resolution in deep regions. Third, more contrasts, including fat cells, can be imaged in vivo by 1046 nm NIR-OR-PAM. In deep regions, 1046 nm NIR-OR-PAM resolved more vascular structures and the images were less blurred than those from 570 nm OR-PAM. Also, 1046 nm NIR-OR-PAM better penetrated through a bleeding area and resolved information underneath it. Our results showed the unique advantages of 1046 nm NIR-OR-PAM and should facilitate the label-free application of near-infrared light in OR-PAM.

8943-192, Session PTues

PDT induced microvacular changes assessed by photoacoustic microscopy

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Photoacoustic Microscopy (PAM) provides noninvasive, high-resolution imaging microvasculature and blood related parameters such as blood volume and blood oxygen saturation in vivo without any contrast agent administration. Most therapies such as photodynamic therapy (PDT) induce changes in the vasculature and these changes can be indicative of therapeutic response. Since the earliest changes occur in the microvasculature, it is imperative to quantify these changes to assess the therapy response earlier. We developed and built a custom fast galvo-scanning based PAM system with ~8 micron lateral resolution for imaging capillaries during vascular targeted therapies.

To demonstrate the feasibility of this system for imaging PDT changes, HPPH photosensitizer was administered via iv injection in a nude mouse and the capillary network in the ear wass imaged before HPPH injection as well as before, during and after PDT. PDT effects at different fluence rates were also investigated. The results show that the system has high sensitivity and resolution in imaging the small vessels, HPPH induced vascular disruption in the capillaries, and the rate of vascular disruption is fluence rate dependent. Thus, the PAM system is sensitive enough to investigate mechanistic studies of vascular targeted therapies such as PDT in the capillary level without imaging contrast agent administration.

8943-193, Session PTues

Bimodal photoacoustic and optically mediated ultrasound microscopy for simultaneous bioimaging of function and structure

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We upgraded the experimental setup that was described here [Pavel Subochev, Alexey Katichev, Andrey Morozov, Anna Orlova, Vladislav Kamensky, and Ilya Turchin // Simultaneous photoacoustic and optically mediated ultrasound microscopy: phantom study //Optics Letters, Vol. 37, Issue 22, pp. 4606-4608 (2012)] and reported at BiOS-2013.

Unfortunately, last year my US VISA was delayed, so I could not make oral presentation personally. This year I would really like to present new results of devoted to in vivo application of our new experimental setup. It is PA microscopy setup with “free” extension to dual-modality PA/US imaging. We use fiber bundle to ensure ring-shaped illumination of the studied tissue, and focused PVDF transducer as a detector.

8943-194, Session PTues

Lifetime-resolved photoacoustic imaging of activatable probes

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Activatable photoacoustic probes have a promising future due to their ability to provide high-resolution, high-penetration depth information on enzyme activity in vivo. Spectral identification methods, however, suffer from heterogeneous optical properties and wavelength-dependent light attenuation in tissue, thereby limiting the effective suppression of background noise signal. Our approach is predicated on probing the excited-state lifetime of a dual-labeled methylene blue (MB) probe that changes its lifetime from short (< 100 ns) to long (70 ?s) upon cleavage. Recently, we have reported on the ability of our system to probe the long lifetime of MB monomers and to differentiate between monomers and dimers based on their lifetime contrast. However no direct measurement of the dimer lifetime was performed due to time resolution limitations. Here we introduce an improvement to our system which significantly increases the system sensitivity to fast changes, and reduces the minimum resolvable lifetime down to a few nanoseconds. We applied this method to probe the excited-state lifetime of a mixed monomer/dimer dye solution immediately after excitation. Preliminary results show signals with a multi-component exponential decay, comprised of both long and short lifetime regions. The long lifetime region is attributed to MB monomers, however further work is needed to identify the component(s) of short lifetime. Our study could provide a new detection method for activatable probes based on the analysis of multicomponent photoacoustic decay, therefore improving background suppression by differentiating the lifetime of the probe and endogeneous tissue absorbers.

8943-195, Session PTues

Feasibility and potential of photoacoustic imaging for the non-invasive molecular profiling of cancer

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Although molecularly targeted cancer therapies have shown great promise, it is now evident that tumour responses are dependent upon the genetic context. Spatial and temporal tumour heterogeneity renders biopsy of solid tumours unsuitable for determining the genetic profile of the disease, making adaptation of appropriate therapy difficult. We have ultilized the tunable optical absorption characteristic of gold nanorods to assess the potential of photoacoustics for non-invasive multiplexed molecular imaging. Gold nanorods with resonance peaks at 700nm and 900nm were functionalised with in-house antibodies ICR55 and ICR62, targeted to HER2 and EGFR transmembrane receptors, respectively. Two human squamous cancer cell lines, (LICR-LON-HN4 expressing high HER2 and low EGFR, and A431 expressing high EGFR and low HER2), were incubated with the targeted nanorods for 24 hours. Cells were then, incorporated as simulated tumours in tissue-like phantoms composed of 7.5% gelatin containing 0.5% Intralipid® for optical scattering and imaged at a depth of 2.5 cm, using a new clinical in-house multi-spectral photoacoustic imaging system. Images were obtained from the cell-inclusions for wavelengths ranging from 700 to 950 nm at 10 nm intervals, and the mean amplitude of the photoacoustic image was computed for each wavelength, to determine their relative receptor expression levels. The molecular profile of the cells obtained using multi-wavelength photoacoustics had substantial similarity to that obtained using flow cytometry. These preliminary results confirm selective uptake of the functionalised nanorods, depending on the cellular expression of therapeutically important oncoproteins, and give an indication of the potential of photoacoustics for multiplexed molecular profiling.

8943-196, Session PTues

Characterisation of contrast agents for photoacoustic imaging

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The efficiency of photoacoustic (PA) signal generation in organic dye based contrast agents used for molecular imaging depends on various factors such as ground state bleaching, fluorescence yield and the photostability of the chromophore. For nanoparticle based agents, optically-induced shape changes and the mismatch in thermodynamic properties with the surrounding tissue can have a profound influence on PA generation efficiency. However, in previous studies, PA contrast agents have been characterised solely on the basis of their optical properties (eg their extinction coefficients), which does not yield information on the PA generation efficiency. To address this we have developed a protocol, based on absolute and relative PA measurements, that determines the efficiency of the conversion of light to acoustic pressure and provides a means of identifying the relevant loss-mechanisms. Using this protocol, we measured the PA generation efficiency as well as other PA properties, such as photostability and the PA spectrum of various commonly used agents (gold nanorods, carbon nanotubes and fluorescent dyes) as well as a variety of novel agents (e.g. polypyrrole nanoparticles and copper sulfide nanoparticles). These were imaged using a tissue phantom to establish the minimum detectable concentration and the penetration depths likely to be achieved in vivo. Finally, a selection of contrast agents were conjugated with different antibodies and their PA properties evaluated to study the effect on generation efficiency. This study provides new criteria for the selection of PA contrast agents which incorporates all the relevant optical and thermodynamic parameters involved in the signal generation process.

8943-197, Session PTues

Photoacoustic molecular imaging of angiogenesis using theranostic ???3-targeted copper nanoparticles incorporating a Sn 2 lipase-labile fumagillin prodrug

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Photoacoustic (PA) tomography imaging is an emerging, versatile, and noninvasive imaging modality, which combines the advantages of both optical imaging and ultrasound imaging. It opens up opportunities for noninvasive imaging of angiogenesis, which is an feature of skin pathologies including cancers and psoriasis. In this study, high-density copper oleate encapsulated within a phospholipid surfactant (CuNPs) generated a soft nanoparticle with PA contrast comparable to gold. Within the NIR window, the copper nanoparticles provided a 6-fold higher signal than that of blood. ???3-integrin targeting of CuNPs in a Matrigel mouse model demonstrated prominent ($p<0.05$) PA contrast enhancement of the neovasculature compared with mice given nontargeted or competitively inhibited CuNPs. Incorporation of an Sn-2 lipase-labile fumagillin prodrug into the CuNPs produced marked antiangiogenesis in the same model, demonstrating the theranostic potential of a PA agent for the first time in vivo. With PA signal comparable to gold-based nanoparticles yet at lower material cost and with demonstrated drug delivery potential, ???3-targeted CuNPs hold great promise for the management of skin pathologies with neovascular features.

8943-198, Session PTues

Concurrent photoacoustic markers for direct three-dimensional ultrasound to video registration

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Fusion of video and other imaging modalities is common in modern surgical procedures to provide surgeons with additional information that can provide precise surgical guidance. An example of such uses interventional guidance equipment and surgical navigation systems to register the tools and devices used in surgery with each other. In this work, we focus explicitly on registering three-dimensional ultrasound with a stereocamera system. These surgical navigation systems often use optical or electromagnetic trackers. However, both of these tracking systems have various drawbacks leading to target registration errors of approximately 3mm. Previous work has shown that photoacoustic markers can be used to register three-dimensional ultrasound with video resulting in target registration errors which are much lower than the current state of the art. This work extends this idea by generating multiple photoacoustic markers concurrently as opposed to the sequential method used in the previous work. This development greatly enhances the acquisition time by a factor equal to the number of concurrently generated photoacoustic markers. This work is demonstrated on a synthetic phantom and an ex vivo porcine kidney phantom. The resulting target registration errors for these experiments ranged from 840 to 1360 um and standard deviations from 370 to 640 um.

8943-199, Session PTues

Gold nanoparticle templated microbubbles for enhanced photoacoustic and ultrasound imaging

Jacob Dove, Todd W. Murray, Mark A. Borden, Univ. of Colorado at Boulder (United States)

Medical imaging contrast agents have improved the ability to detect and treat diseased tissue. Two agents that have seen widespread use for contrast enhanced imaging are plasmonic nanoparticles for photoacoustics and microbubbles for ultrasound. Nanoparticles offer enhanced photoacoustic contrast due to their strong optical absorption, and microbubbles increase ultrasound contrast through efficient scattering around resonance. We report on a novel gold nanoparticle-coated microbubble (AuMB) capable of enhancing both ultrasound and photoacoustic contrast. AuMBs were comprised of 5 nm gold nanospheres coated to the surface of size selected, lipid-encapsulated microbubbles through a biotin-avidin coupling scheme. Nanoparticle surface density was controlled through the amount of biotinylated lipid incorporated into the microbubble shell. Upon illumination with a pulsed laser source, solutions of AuMBs produced a much larger photoacoustic response than solutions of nanoparticles alone. Ultrasound and photoacoustic images of an agarose flow-through tissue phantom were acquired. AuMBs solutions produced strong contrast in both the ultrasound and photoacoustic images. The photoacoustic response of single AuMBs was studied using a modified optical microscope, in which an individual AuMB was excited with a pulsed laser and the bubble wall radius was tracked using light scattering. It was found that pulsed laser illumination caused individual AuMBs to be driven into resonance, potentially allowing for efficient photoacoustic emission at the resonance frequency and producing the signal enhancement observed in the AuMB solutions.

8943-200, Session PTues

Photoacoustic phasoscopy super-contrast imaging correlating optical absorption and scattering

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Photoacoustic imaging is attracting significant research interest due to its breakthrough of optical diffusion limit by “listening to photons”. Dual-modal imaging approaches combining PA with other optical imaging modalities have been proposed to give complementary images by collecting both photoacoustic wave and scattered photons. However, few research has been done to explore the intrinsic correlation between endogenous photoacoustic wave and scattered photons coming from the same object.

Phasoscopy is a recently proposed concept by our group [Appl. Phys. Lett., 101, 043702 (2012)], correlating electromagnetic (EM) absorption and scattering from the same object based on energy conservation. Phase information can be extracted from EM absorption induced acoustic wave and scattered EM wave, which then can be used to characterize the different biological tissues in higher sensitivity and selectivity than existing approaches. In this paper, a novel imaging modality, termed photoacoustic phasoscopy imaging, is proposed and verified experimentally based on phasoscopy with pulsed laser illumination. Both endogeneous photoacoustic wave and scattered photons are collected simultaneously to extract the discriminative phase information. Photoacoustic phasoscopy imaging results on vessel-mimicking phantom and ex vivo porcine tissues demonstrated >10 times improvement on image contrast than conventional photoacoustic imaging based on optical absorption only. Moreover, photoacoustic phasoscopy imaging is inherently immune to the laser and system variations because when input laser intensity fluctuates, both the photoacoustic intensity and scattered photons intensity fluctuate in the same way, leading to unchanged phase. In vivo 3D imaging results will be demonstrated in near future for promising clinical applications.

8943-201, Session PTues

Photoacoustic active ultrasound element for catheter tracking

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In recent years various methods have been developed to improve the ultrasound based interventional tool tracking and guiding. Some of these methods are based on the active ultrasound catheter approach, including an active ultrasound element which transmits ultrasound pulses through the catheter. The active elements are generally made of piezoelectric materials however in some applications driving high voltage to the piezo element raises safety concerns. In addition the metallic electrical wires of the piezo element may also cause artifacts in CT and MR imaging. In this work we focus explicitly on developing an active ultrasound element based on photoacoustic (PA) effect to overcome these issues. The element is composed of a pulsed laser light, optical fibers and PA targets. In our approach we have used an absorbing liquid as the PA target in order to eliminate the ablation issue of solid materials due to high laser energy. Therefore in this method the photoacoustic generation is based on the liquid phase change or vaporization effect which generates significantly higher signal amplitude than the traditionally used mechanism of thermal expansion. We have made the prototype catheter and performed the phantom experiments. The preliminary results showed that the PA element is able to generate high intensity broadband ultrasound waves with driving laser pulse energy less than 50uJ. The emission profile can be controlled by changing the fiber size and optical properties of the liquid target. We were able to track the prototype catheter using the ultrasound pulses generated by the photoacoustic active element.

8943-202, Session PTues

Wide-field imaging through turbid media

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Optical spectroscopy and imaging of tissue provide tremendous amounts of information that can be utilized for both diagnostic and therapeutic purposes. One critical limitation of optical tools, however, is the depth for which optical information can be reliably delivered or retrieved. This ability is severely hampered due to the multiple scattering, mainly due to inhomogeneous refractive index distribution. One of the approaches to suppress multiple light scattering involves suppressing sample turbidity using wavefront shaping [1], which provides a high degree of control over the scattered light in, e.g., space [2], time [3], wavelength [4], polarization [5], and transmitted energy [6].

Optical phase conjugation (OPC) is another technique to reverse the effects of multiple light scattering [7]. The first demonstrations of turbidity suppression in biological samples used photorefractive crystal-based OPC setups [8, 9]. Recent trends in the field involves digital implementation of OPC, referred as to digital optical phase conjugation (DOPC) [10]. The implementation of DOPC, however, poses challenging alignment issues since the wavefront sensor and actuator are decoupled in space. Here, we report the implementation of wide-field DOPC [11], where the challenging issue of aligning the wavefront sensor and actuator

has effectively been solved, by employing a Sagnec ring interferometry. The SLM used as wavefront "actuator" is directly projected onto the CCD used as "sensor", the key to the sub-wavelength accuracy attained in the optical alignment. Our work provides a robust approach to implement a full-field DOPC system for controlling light propagation through complex media such as human tissue.

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8943-203, Session PTues

Aggregate enhanced trimodal porphyrin shell microbubbles for ultrasound, photoacoustic and fluorescence imaging

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Microbubbles are currently clinically used as ultrasound contrast agents to provide acoustic contrast between blood vessels/pools and soft tissue and have also been developed as delivery vehicles for site-specific ultrasound triggered drug and gene delivery. Using a MB as a drug delivery vehicle not only enables localized delivery but also enhances drug delivery as the bursting of the MB with ultrasound transiently increases cell membrane and blood vessel permeability, which has been shown to increase local drug delivery. However, bursting of the microbubble causes the gas to diffuse out of the microbubble, thereby eliminating the ultrasound signal generation and an inability to track the fate of the microbubble. Here we introduce intrinsically trimodal microbubbles with ultrasound, photoacoustic and fluorescence properties. These trimodal microbubbles possess an acoustic resonance frequency at 4.5MHz, optical absorption at 824nm and fluorescence emission in the near-infrared region. These photonic microbubbles provide a unique opportunity to combine specific microbubble behavior (ultrasound-triggered bursting) with high-resolution photoacoustic imaging and fluorescence imaging to monitor the microbubbles before and after drug delivery. We investigate the acoustic and optical properties both before and after bursting to demonstrate that upon bursting of these microbubbles, despite the elimination of ultrasound signal, the resulting nanostructures can be tracked using optical means. These trimodality microbubbles may have broad image-tracking implications for therapeutic applications involving microbubble bursting.

8943-204, Session PTues

Developing photoacoustic tomography system with the aid of the acoustically penetrable optical reflector

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In order to avoid the optical blockage by opaque ultrasound transducer in photoacoustic tomography system, we provide a novel concept to using a specific material which can reflect light but allow the transmission of ultrasound. This material, we called Acoustically penetrable optical reflector (APOR), can be a plastic membrane with a metal coating. By putting the APOR in front of the transducer, one can feasibly manipulate the light illumination pattern independently without affecting the ultrasonic detection.

8943-205, Session PTues

Photothermal bleaching and recovery analysis in photoacoustic microscopy

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Fluorescence recovery after photobleaching is a widespread method for investigating molecule dynamics in cells. Photoacoustic microscopy (PAM) can provide dynamic information in a similar way while possessing some different characteristics. In photoacoustic recovery after photothermal bleaching (PRAP), a high-energy laser beam is firstly used to photothermally bleach the light-absorbent particles. Then the change of the photoacoustic signal in this bleached region is measured by the same but attenuated laser beam. The signal change shows a recovery profile because of the renewal of fresh nanoparticles from the surrounding unbleached region. By analyzing the profile, the characteristics of the particle and embedding medium can be revealed. The photothermal bleaching of gold nanoparticles in PAM behaves quite differently before and after the absorbers are raised to a critical temperature by the excitation laser pulses. The nanoparticles can be easily photothermally bleached with a relatively high intensity laser, and readily monitored with negligible bleaching at the stage of recovery using sub-threshold laser pulse energy. In this study, PRAP is demonstrated first in a simple phantom study, showing the solution viscosity change when glycerol concentration in water is increased. Then measurement involving live cells loaded with gold nanoparticles indicates the flowability of cytoplasm. Cell staining was performed after the photoacoustic experiment to evaluate the cell viability, and the result showed acceptable (>50%) cell viability.

8943-206, Session PTues

Ultrasound modulated fluorescence based on fluorescent microbubbles

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In this research, we designed a fluorophore labeled microbubbles as contrast agent to study ultrasound modulated fluorescence (UMF) phenomenon and its modulation efficiency. Microbubbles labeled with different concentrated fluorophores were studied. Self-quenching occurred when the surface fluorophore density is relative high, as presented by the lifetime measurement. The UMF signal was measured on individual microbubbles under ultrasound pulses. The UMF signal was compared with the microbubbles' size oscillation and showed temporal synchronization. Our results presented a statically significant correlation between the modulation efficiency and the surface fluorophore density. Further, under varied acoustic pressures, the modulation efficiency showed strong dependence on the oscillation amplitude. In the end, a fluorophore-quencher-labeled microbubble system was studied and the UMF signal from both donor and quencher were presented.

8943-207, Session PTues

Ultrahigh-resolution photoacoustic microscopy via modulation of a pulsed laser source

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Recent advances in photoacoustic microscopy (PAM) using transient absorption have enabled subcellular transverse and axial resolutions through optical sectioning. This technique, called transient absorption ultrasonic microscopy (TAUM), is based on pump-probe spectroscopy and required the setup and maintenance of co-propagating pump and probe beams carefully aligned with a set time delay. The pump and probe were modulated at different frequencies enabling the detection of the pump-probe (optically sectioned) signal at the sum and difference frequencies. Here we describe a more efficient design that uses a single modulated beam to obtain the same subcellular resolution. The introduction of a modulation frequency on the pulsed laser source frequency encodes the PAM signal at the fundamental frequency and the TAUM signal at the second harmonic of the modulation. The TAUM signal is shifted to the second harmonic because it is a two photon process. This design is conceptually equivalent to setting the time delay between the pump and probe to zero and modulating both at the same frequency in our previous system design. This modification allows for collection of photoacoustic images with optical sectioning in both the axial and transverse imaging planes with the simple addition of a modulation frequency to the optical pathway of an existing PAM system. The imaging capabilities of this system are validated by capturing a 3-D volume of individual erythrocytes in a blood smear with an axial resolution of 1.5 µm and lateral resolution of 1 µm.

8943-208, Session PTues

Real-time photoacoustic imaging with an acoustic camera using 2D-optical ultrasound detection

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One important requirement on imaging systems for medical and biological applications is the possibility to perform in-vivo experiments. Therefore, the imaging time has to be as short as possible. In this work we present a real-time photoacoustic imaging setup that allows recording of C-scan images with a frame rate of 10 Hz.

The acoustic camera setup can be separated into two main parts. The first part contains a 4f-acoustic lens, which creates an acoustic image of the initial three-dimensional acoustic pressure distribution outside the sample in an accessible volume in a water tank. The second part contains the 2D-optical ultrasound detector. It uses a CCD-camera to record intensity modulations caused by the pressure induced change of the optical reflectance at a glass-liquid interface. To increase the detection sensitivity compared to previous setups we have replaced water at the detection interface by a liquid with a 2.5 times higher elasto-optic coupling coefficient and we use a cooled low-noise 14bit CCD-camera to record the images. Unit magnification in axial and lateral direction of the imaged pressure distribution is obtained by the 4f-acoustic lens system. Due to that the 3D-image can be directly composed from C-scan images recorded while scanning the sample in axial direction or time delay scanning relating to the excitation laser pulse.

The proposed method directly provides 2D images of absorbing structures without the need of computational reconstruction algorithms.

Experiments are performed using phantom samples which mimic the properties of biological samples to investigate resolution, sensitivity and the overall applicability of this technique for real-time photoacoustic imaging.

8943-209, Session PTues

Handheld probe combining laser diode and ultrasound transducer array for ultrasound/ photoacoustic imaging

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Ultrasound and photoacoustics can be utilized as complementary imaging techniques to achieve more valuable clinical diagnosis. Photoacoustic provides optical contrast and functional information while ultrasound provides mechanical and anatomical structure. As of yet, photoacoustic imaging uses large and expensive systems which limit their clinical application and make the combination costly and impracticable. In this work we present and evaluate a compact and ergonomically designed handheld pulsed laser probe connected to a portable ultrasound system for inexpensive, real-time dual modality ultrasound/photoacoustic imaging. The probe integrates an ultrasound transducer array and a highly efficient diode stack laser emitting 100 ns pulses at 800 nm wavelength, with high frequency repetition rate up to 10 kHz. The diodes are driven by an electrical driver developed for very short high power pulses, triggered externally with high stability to synchronize the ultrasound scanner and laser pulsing. The emitted beam is collimated and shaped with diffractive optical elements (DOE) system delivering a homogenized rectangular laser beam energy distribution of 0.5 mJ per pulse. The system performance is tested using tissue-mimicking phantoms and the feasibility of in-vivo measurements is demonstrated.

8943-210, Session PTues

Accuracy of inversion schemes for multiwavelength quantitative photoacoustic imaging

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Quantitative photoacoustic imaging (QPAI) aims to obtain images of chromophore concentrations, or a related quantity such as blood oxygenation, by performing a spectroscopic optical inversion on multiwavelength initial pressure distributions. It is a complex nonlinear inverse problem due to the non-uniform and wavelength dependent light fluence, and the light model used, as well as errors in the initial pressure distributions, will impact on the accuracy of the concentration estimates. A realisable solution to QPAI would facilitate accurate, quantitative, high resolution 3D functional and molecular imaging. The QPAI inversion has previously been tackled using a range of approaches, from a linear inversion, which ignores the effect of the fluence and is therefore inaccurate, to optimisation-based approaches with accurate light models, which can be excessively computationally intensive. Methods that lie between these extremes, that improve on the accuracy of the linear inversion without the computational expense of a full model-based inversion, have been proposed but are typically accurate only for a limited range of circumstances or parameters. In this paper we test several of these schemes in order to determine when they are valid. The effect on the accuracy of the inversions due to the approximations in the fluence models used, and due to assumptions in the acoustic inversion (image reconstruction) will be described. A detailed analysis of the sources of error in QPAI schemes, and their effects on estimates of chromophore concentrations and blood oxygenation, is crucial for the practical application of QPAI in clinical and preclinical medicine.

8943-211, Session PTues

In vitro photoacoustic detection of hemoglobin oxygen saturation variation using a pulsed broadband soupercontinuum laser source

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We have utilized a pulsed broadband supercontinuum laser to research a functional photoacoustic (PA) imaging in phantoms and bovine bloods. By using a supercomtinuum laser, tunable band pass filter, and transmission type PA system, we observed variation of the PA signals at various concentrations of inks and bloods. The supercontinuum laser composed of a Q-switched Nd: YAG microchip laser and photonic crystal fiber (PCF). The measured optical spectrum and pulsed energy are from 600 to 1700 nm and 500 nJ, respectively. The tunable bandpass filter was implemented in a transmission type to increase the laser output power after filtering by minimizing light reflection. We selected two optical broadband wavelengths (band1: 500-560 nm, band2:560-660 nm). The filtered light is focused on the sample, and then a transducer detected the PA signal in transmission mode. Then, two quantitative experiments and analysis were processed: (1) estimating the total concentration of the mixture of red and blue inks and the ratio of the red ink concentration to the total ink concentration and (2) calculating the relative the concentration of total hemoglobin (HbT) and oxygen saturation of hemoglobin (SO2) of bovine blood in vitro. The results indicates that the PA imaging used the broadband pulsed lasers potentially can provide the functional PA information of HbT and SO2.

8943-212, Session PTues

Combined optical and mechanical scanning in optical-resolution photoacoustic microscopy

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Optical-resolution photoacoustic microscopy (OR-PAM), based on the photoacoustic effect, detects optical absorption contrasts with the highest sensitivity of all optical imaging modalities. The high detection efficiency and resolution of intrinsic optical absorbers (e.g., oxy-hemoglobin (HbO2) and deoxy-hemoglobin (HbR)) enable OR-PAM to generate label-free functional images of fine structures. OR-PAM has already found broad biomedical applications in neurology, ophthalmology, and vascular biology. However, with current OR-PAM system, either the slow imaging speed hinders observation of fast dynamic activities in vivo; or the limited imaging modes and functions restrict its usage in in vivo functional imaging.

Here, we report a combined optical and mechanical scanning (COM) in OR-PAM, which provides five scanning modes with fast imaging speed and wide field of view (FOV). With two-dimensional (2D) galvanometer-based optical scanning, we have achieved a 2 KHz B-scan rate and 50 Hz volumetric-scan rate, which enables real-time tracking of cell activities in vivo. With optical-mechanical hybrid 2D scanning, we are able to image a wide FOV (12?12 mm2) within 3 minutes, which is 20 times faster than the conventional mechanical scan in our second-generation OR-PAM. With three-dimensional mechanical-based contour scanning, we can maintain the optimal signal-to-noise ratio and spatial resolution while imaging objects with uneven surfaces, which is ideal for fast and quantitative studies of tumors and the brain. COM-OR-PAM has been experimentally demonstrated to be a powerful tool for both real-time cell tracking in vivo and fast wide-FOV monitoring of tumor neovascularization and metabolic activity.

8943-213, Session PTues

Model-based tomographic optoacoustic reconstructions in acoustically attenuating media

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Acoustic attenuation influences the transmission of the ultrasonic waves excited optoacoustically in biological samples, in a way that the amplitude of the waves is reduced as they propagate through acoustically attenuating tissues. Furthermore, being dependent on frequency, acoustic attenuation causes also broadening of the time-resolved optoacoustic signals, which in turn leads to blurring of features and structures in the images. The effects of acoustic attenuation are more prominent for the high frequency components of the optoacoustic waves, so that they must be taken into account for high resolution imaging. However, most reconstruction algorithms used in optoacoustic tomography do not account for attenuation of optoacoustic waves. In this work, we modify a model-based reconstruction algorithm developed in our group to incorporate the effects of acoustic attenuation in tomographic optoacoustic imaging setups. As the waves propagate through the sample and the coupling medium (water), the undergone attenuation is space and frequency dependent, and the model-based approach allows effectively accounting for this dependency. The results obtained showcase a good performance of the introduced method in terms of image quality and resolution improvement.

8943-214, Session PTues

Quantification of optical attenuation coefficient based on continuous wavelet transform of photoacoustic signals measured by a focused broadband acoustic sensor

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We proposed a method to quantify optical attenuation coefficients of optical absorbers in which the continuous wavelet transform was used to calculate the time-resolved frequency spectra of photoacoustic (PA) signals. The instantaneous frequencies were calculated from the time resolved frequency spectra of the PA signals. In previous report, we demonstrated that the optical attenuation coefficients of the optical absorbers could be quantified from the peaks of the instantaneous frequencies because the peaks of the instantaneous frequencies linearly related to optical attenuation coefficients of optical absorbers (Hirasawa et al, Proc. of SPIE, 85814J, 2013). This method can be used to monitor absolute oxygen saturation of blood in blood vessels with several millimeter diameters. However, since instantaneous frequencies of PA signals depended on not only optical attenuation coefficients but also blood vessel diameters, the proposed method was applicable to blood vessels with known diameters.

The absolute oxygen saturation of blood was quantified without dependence on the blood vessel diameters by detecting PA signal from small region in comparison with blood vessel. We fabricated the focused broadband acoustic sensor made of P(VDF-TrFE) film to detect PA signals from small region. We experimentally tested effectiveness of the focused acoustic sensor using blood vessel phantoms with diameters ranging 2.0 to 6.0 mm, and optical attenuation coefficients ranging 10 to 30 cm-1. PA signals measured by both the focused acoustic sensor and an unfocused acoustic sensor were compared based upon dependences of instantaneous frequencies on phantom diameters.

8943-215, Session PTues

Simultaneous reconstruction of absorbed optical energy density and speed of sound in photoacoustic computed tomography

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An important and interesting question in photoacoustic computed tomography (PACT) is whether the absorbed optical energy density distribution, A(r), and the speed of sound distribution, c(r), can both be accurately determined from the measured photoacoustic data. It has been demonstrated that incorporating c(r) into PACT image reconstructions can improve the image quality of the reconstructed A(r). However, in many cases c(r) is unknown or cannot be accurately estimated. Therefore, it would be practically beneficial if A(r) and c(r) can be simultaneously reconstructed from the measurements. In this work, we develop and investigate an optimization approach to the simultaneous reconstruction of A(r) and c(r) in PACT. The method is based on an alternating optimization scheme, where A(r) is reconstructed by use of a previously-developed full-wave iterative method, while c(r) is reconstructed by use of a nonlinear optimization algorithm based on the Fréchet derivative of an objective function with respect to c(r). We also give a heuristic necessary condition for the accurate reconstruction of c(r). Computer simulations are employed to assess the accuracy and robustness of the alternating optimization method, as well as verify the heuristic argument. The numerical results show that, in some cases, it is possible to achieve accurate simultaneous reconstruction of both properties when the heuristic condition is met by incorporating a regularization term into the reconstruction algorithm. Examples of cases in which accurate simultaneous reconstruction is not possible are also provided. Even when c(r) cannot be accurately reconstructed, we demonstrate that A(r) can be more accurately reconstructed by this method than when c(r) is assumed homogeneous.

8943-216, Session PTues

Freehand spatial-angular compounding of photoacoustic images

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Photoacoustic (PA) imaging is an emerging medical imaging modality that relies on the absorption of optical energy and the subsequent emission of an acoustic wave. PA images are susceptible to background noise artifacts that reduce the Signal-to-Noise ratio (SNR) and Contrast-to-Noise ratio (CNR). Spatial Compounding is a well-known noise reduction technique for ultrasound imaging. In this study, we implemented freehand spatial-angular compounding of PA images to enhance SNR and CNR.

Spatial-angular compounding requires multiple PA images in similar elevational planes acquired at varied elevational angles. An external tracker system is used to provide the pose information of each PA image. Based on the pose information, frames in similar elevational planes are filtered from the collected pre-beamformed RF data. These selected frames were compounded with two compounding methods (Averaging and Selective Averaging) applied independently to pre-beamformed RF, beamformed RF, and envelope-detected PA data, resulting in six different compounded image combinations. The more conventional Averaging method applies the mean operation to all filtered frames. Selective-Averaging is a dynamic compounding method that uses image content information to sum overlapping regions of PA signal, while applying conventional averaging to the remaining regions. This method is applied once to each incoming frame and the dynamically updated compounded image.

Compounded PA images from each of the six compounding pipelines have higher CNR and SNR than a single PA image, while the Selective-Averaging method applied to envelope-detected data has the highest CNR and SNR.

8943-217, Session PTues

Photoacoustic measurement of stochastic microstructure using the spectral parameter

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The microstructure of biological tissue is related with the types of the tissues and can be an important indicator for the pathological changes of the tissues. So it would be very useful to quantitatively measure the stochastic microstructures having dimensions of dozens of microns inside turbid media for biomedical applications, such as the early diagnosis and staging of cancer, blood testing, and detection of cholelithiasis. Photoacoustic signals are related with the microstructure of tissue. But for the time domain of the photoacoustic signals, there is still a hard problem to quantitatively detect the size of the microstructure for the limit of the image depth in tissue owning to the strong attenuation of the high frequency. Spectral properties of the photoacoustic signal can also reflect the microstructure even in the low frequency region. So this provides a possible to measure the microstructure of the tissue in a spectral method.

In this study, a theoretical derivation demonstrates that there is a one-to-one correspondence between the spectrum parameter extracted from photoacoustic signal and the effective diameters of absorbers. And a calibration method is adopted to remove the exterior influent factors which would affect the spectrum parameter such as the

instrument system setting, etc. Based on this theory, we have measured the dimensions (49um, 94.8um and 199um) of the micro-particles stochastically distributed in the deep turbid phantoms within the relatively low frequency domain. Since microstructures in tissue are closely related to physiological and pathological processes of an organism, the proposed method may provide a potential biomedical application.

8943-218, Session PTues

Real-time photoacoustic and ultrasound parallel imaging system facilitated by GPU acceleration and code optimization

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A highly optimized back projection (BP) algorithm for photoacoustic tomography (PAT) imaging is developed on the latest graphics processing unit (GPU). In photoacoustic (PA) imaging, the BP method is the classical and standard approach for 2-D and 3-D biomedical image based detection and therapy. However, the use of BP algorithms to reconstruct PA images is limited by their long computation time. Despite of its parallel nature, optimization of the BP algorithm on a GPU has been shown to be a challenge, particularly when real-time image reconstruction is required or the size of the reconstructed image is large. This paper proposes an optimized algorithm based on the BP method that can be adapted to a high level paralleled GPU to reconstruct PAT images in real-time. With parallel acceleration of the GPU, the computation time to reconstruct one PAT image decreases by 99.8%. The reconstruction algorithm was implemented on a GPU installed in a Mac Pro with real time data feeding from a Verasonic ultrasound platform. The system acquires and displays both US and PA images alternately at 10 frames per second, which is limited by the repetition rate of the laser illumination for PA imaging but still improvable. The system is validated by phantom study and in vivo experiments on human joints.

8943-219, Session PTues

Functional connectivity in the mouse brain imaged by B-mode photoacoustic microscopy

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The increasing use of mouse models for human brain disease studies, coupled with the fact that existing functional imaging modalities cannot be easily applied to mice, presents an emerging need for a new functional imaging modality. Utilizing acoustic-resolution photoacoustic microscopy (AR-PAM), we imaged spontaneous cerebral hemodynamic fluctuations and their associated functional connections in the mouse brain. The images were acquired noninvasively in B-scan mode with a fast frame rate, a large field of view, and a high spatial resolution. At different locations relative to the bregma, 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 — the olfactory bulb, limbic, parietal, somatosensory, retrosplenial, visual, motor, and temporal regions —were studied up to several millimeters in depth. The locations of these regions agreed well with the Paxinos mouse brain atlas. The B-scan functional connectivity maps at different brain locations can be combined to form a three dimensional correlation map of the entire mouse brain. This 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 microscopy is able to detect connectivities between different functional regions in B-scan mode, promising a powerful functional imaging modality for future brain research.

8943-220, Session PTues

64-line-sensor array: Fast imaging system for photoacoustic tomograph

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Three-dimensional photoacoustic tomography with line sensors, which integrate the pressure along their length, has shown to produce accurate images of small animals. To reduce the scanning time and to enable in vivo applications, a detection array is built consisting of 64 piezoelectric line sensors which are arranged on a semi-cylinder. When measuring line integrated pressure signals around the imaging object, the three-dimensional photoacoustic imaging problem is reduced to a set of two-dimensional reconstructions and the measurement setup requires only a single axis of rotation.

The shape and size of the array were adapted to the given problem of biomedical imaging and small animal imaging in particular. Detailed simulations regarding the dimensions and electrical characteristics of the lines and the shape of the array were performed. In particular, the length and width of individual line elements had to be chosen in order to take advantage of the favorable line integrating properties, maintaining the requested resolution of the image. For data acquisition the signals from the 64 elements are amplified and multiplexed into a 32 channel digitizer. Single projection images are recorded with two laser pulses within 0.2 seconds, as determined by the laser pulse repetition rate of 10 Hz. Phantom experiments are used for characterization of the line-array. The ongoing research focuses on biomedical imaging of inner organs of small animals.

Compared to previous implementations with a single line sensor scanning around an object, with the developed array the data acquisition time can be reduced from about one hour to about one minute.

8943-221, Session PTues

Gold nanorods combine photoacoustic and Raman imaging for detection and treatment of ovarian cancer

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Gold nanorods (GNRs) were synthesized with surfactant templating and coated with IR792 to produce surface-enhanced Raman signal (SERS). Subcutaneous and orthotopic tumor models were created in nude mice using the OV2008 cell line, and a Nexus128 scanner from Endra LifeSciences was used to collect the photoacoustic data. We used GNRs with resonance at 756 nm, and the Raman signal was 10-fold larger than 60 nm gold core/silica shell nanoparticles. This signal was stable for over 24 hours in 50% serum. The batch-to-batch reproducibility was 15.5% and 3.6% in the SERS and photoacoustic modalities for n=4 batches. Animals were injected with 200 µL of 2.5, 5.4, and 16.8 nM GNRs. Relative to baseline photoacoustic signal, these concentrations increased tumor signal 1.3-, 1.6-, and 2.5-fold, respectively. The maximum signal increase occurred within 2 hours of injection and persisted for at least 24 hours ($p<0.05$ for at least 3 animals). Assaying for gold in the tumors validated signal—we found a strong correlation ($R2>0.90$) between tumor gold concentration and photoacoustic signal. By 24 hours, free GNRs had been sequestered to the liver and spleen with 2%ID/g immobilized in the tumor. The same GNRs produced SERS signal, and Raman maps were created with least squares analysis. We used the Raman signal to identify tumor margins and also to monitor resection and ensure complete removal of tumor tissue. Thus, the GNRs allow pre-surgical photoacoustic visualization for tumor staging and intra-operative Raman imaging to guide resection. Future work will study GNRs targeted to cell surface proteins to increase tumor accumulation.

8943-222, Session PTues

Identification of red blood cell rouleaux formation using photoacoustic ultrasound spectroscopy

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Red blood cells (RBCs) can aggregate in a face-to-face stacked linear arrangement called rouleaux. Rouleaux formation is a reversible phenomenon that occurs during low blood flow and small shearing forces in circulation. Certain pathological conditions can alter the molecular constituents of blood and properties of the RBCs leading to enhanced rouleaux formation, which results in impaired perfusion and tissue oxygenation. Current techniques for assessing RBC aggregation rely on rheological methods such as viscometers that are dependent on the shearing geometry and the experimental protocol. Rouleaux were artificially generated using Dextran-70 and examined using a photoacoustic microscope, Kibero (Saarbrücken, Germany). Single rouleaux were irradiated with a 532 nm pulsed laser focused to a 10 ?m spot size, and the resulting photoacoustic signals recorded with a 200 MHz transducer. The laser and transducer were co-aligned, with the sample positioned between them. The frequency-domain photoacoustic ultrasound spectra were calculated where a single spectral minimum at 266 ± 7.1 MHz was observed for rouleaux with lengths ranging from 10 to 20 ?m. The spectral minima were in good agreement with a theoretical thermoelastic expansion model using a cylindrical absorber, bearing a diameter equivalent to an average human RBC (7.8 ?m). Predictions of finite element models show that by increasing the spot size to irradiate one entire rouleau, the aggregate length can be estimated from changes in the photoacoustic ultrasound spectral features. These results suggest that photoacoustic ultrasound spectroscopy can be potentially used as a tool for monitoring blood samples for the presence of rouleaux.

8943-223, Session PTues

Controlled surface modification and conjugation of nanoparticles for optoacoustic contrasting

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We present optoacoustic sensing experiments using biosensor NanoLISA and nanoparticle based contrast agents, both developed in our company. The sensing contrast agents were designed for maximum optoacoustic signal. Our optimization was based on physics of optoacoustic generation in plasmonic nanoparticles and published methods for the synthesis and surface modifications of gold nanorods (GNR) and hollow gold nanoshells (HGNS) by pegylation, conjugation and silicanization. Silica coated GNR and HGNS possess significantly enhanced optoacoustic efficiency compared with bare pegylated nanoparticles and OA efficiency is strongly dependent on the thickness of silica layer. For optoacoustic sensing application we employed controlled agglomeration between nanoparticle conjugated with specific antibodies and molecules of protein antigens. This agglomeration produces a shift of plasmon resonance peak as a function of concentration of targeting nanoparticles and protein targets. While a strong agglomeration was observed in the course of interactions between targeted gold nanoparticle conjugates and their antigens, no agglomeration was observed in the case of nonspecific binding. Our experimental results suggest that the effect of controlled agglomeration can be used to determine concentration of different proteins for purposes of in vitro diagnostics

8943-224, Session PTues

Investigation of effective system designs for transcranial photoacoustic tomography of the brain

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Photoacoustic computed tomography (PACT) holds great promise for transcranial brain imaging. However, the strong reflection, scattering and attenuation of acoustic waves in the skull present significant challenges to developing this method. We report on a systematic computer-simulation study of transcranial brain imaging using PACT. The goal of this study was to identify effective imaging system and reconstruction algorithm designs that can be translated for clinical use. The propagation of photoacoustic waves through a model skull was studied by use of an elastic finite-difference time-domain (FDTD) method. The acoustic radiation pattern from a point source just beneath the skull was transcranially observed with a ring transducer array that was level with the point source. The observed radiation pattern was found to contain more of the waves that came through the skull in the form of shear waves than the waves that came in the form of longitudinal waves. This observation revealed that the detection system should capture photoacoustic signals that travel through the skull in the form of shear waves rather than in the form of longitudinal waves. Based on such a system design, reconstruction algorithms, i.e. a back-projection (BP) and an iterative algorithm based on the full wave-equation, were compared in terms of image quality and computational efficiency. This study provides an insight into the wave phenomena in transcranial PACT imaging, as well as a concrete detection design strategy that maximizes image contrast and resolution.Photoacoustic computed tomography (PACT) holds great promise for transcranial brain imaging. However, the strong reflection, scattering and attenuation of acoustic waves in the skull present significant challenges to developing this method. We report on a systematic computer-simulation study of transcranial brain imaging using PACT. The goal of this study was to identify effective imaging system and reconstruction algorithm designs that can be translated for clinical use. The propagation of photoacoustic waves through a model skull was studied by use of an elastic finite-difference time-domain (FDTD) method. The acoustic radiation pattern from a point source just beneath the skull was transcranially observed with a ring transducer array that was level with the point source. The observed radiation pattern was found to contain more of the waves that came through the skull in the form of shear waves than the waves that came in the form of longitudinal waves. This observation revealed that the detection system should capture photoacoustic signals that travel through the skull in the form of shear waves rather than in the form of longitudinal waves. Based on such a system design, reconstruction algorithms, i.e. a back-projection (BP) and an iterative algorithm based on the full wave-equation, were compared in terms of image quality and computational efficiency. This study provides an insight into the wave phenomena in transcranial PACT imaging, as well as a concrete detection design strategy that maximizes image contrast and resolution.

8943-225, Session PTues

Full visibility photoacoustic imaging system using all-optical planar sensor arrays

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In recent years, a range of ultrasonic sensor configurations have been developed to record photoacoustic (PA) signals. The Fabry-Perot (FP) polymer film sensor benefits from being able to detect using small element sizes (10's mu m) with a low noise equivalent pressure (~0.21kPa). The small element size gives benefits in terms of lateral

resolution in the final image. FP sensor arrays are typically designed to be planar, for simplicity of manufacture and interrogation. However, planar sensors have a limited view of the acoustic field, so some of the waves emitted from the PA source are not recorded. The lack of a complete data set results in artifacts in the reconstructed image. Removal of these artifacts is important for image quality, and in particular for quantitative multiwavelength photoacoustic imaging, as the artifacts will change with wavelength.

In this paper we describe a new approach in which the effective detection aperture is increased by using multiple planar arrays and reflectors so that all points in the image volume meet the visibility condition. This results in a significant reduction in artifacts, increased spatial resolution and SNR compared to the limited view planar detection geometry. Several proposed arrangements were assessed to select the optimal configuration. Initial results from an experimental system implementing this design are reported. These developments will lead to a 3D full-visibility PA scanners for whole body small animal imaging with high image fidelity, which will facilitate accurate quantitative photoacoustic imaging.

8943-226, Session PTues

Fiber-based remote photoacoustic imaging utilizing a Mach Zehnder interferometer with optical amplification

Armin Hochreiner, Johannes Bauer-Marschallinger, Peter Burgholzer, Thomas Berer, RECENDT GmbH (Austria)

Remote (or non-contact) photoacoustic imaging techniques (rPAI) allow measurement of photoacoustic signals without the need of physical contact to the specimen. For some applications, like burn diagnostics or imaging during brain surgery, contacting means should be avoided and non-contacting means may provide sterile alternatives. In rPAI photoacoustically generated ultrasonic displacements are detected without physical contact to the sample by utilizing laser interferometric techniques.

In this work we present a remote imaging setup based on an optical fiber-based Mach-Zehnder interferometer. Using optical wave guide technology usually used in telecommunication industries guarantees long life times, simple setup, and relatively low costs. A detection beam is transmitted through an optical fiber to a lens system and is focused onto the surface of the specimen. The back reflected light is collected by the same lens system and coupled into the same optical fiber. To achieve a high signal/noise ratio the reflected light is amplified by means of optical amplification with an erbium doped fiber amplifier before demodulation. Demodulation is done with a custom built balanced photodetector. For image reconstruction a Fourier domain technique is used.

We demonstrate non-contact imaging on tissue mimicking phantoms and on biological samples. Furthermore, we discuss laser safety and analyze the minimum detectable pressure obtainable with the system. The analysis shows that the minimum detectable pressure is about 35Pa for a detection bandwidth of 5MHz. The detection bandwidth can be increased up to 50MHz at the cost of losing sensitivity.

8943-84, Session 13

Photoacoustic tissue characterization using signal envelope statistics and ultrasonic spectral parameters

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Photoacoustic images are useful in determining vascular/gross anatomical structures. When the imaging resolution-volume contains many sub-resolution sources, it can be difficult to quantify changes due to tissue structure/composition solely on image intensity. Ultrasonic-basckscatter signal-envelope-amplitude has a statistical distribution affected by changes in scatterer properties and spatial distribution, while size/concentration can be extracted from the signal's frequency content. Such changes can be quantified by investigating signal-envelope-amplitude fits to theoretical probability-density-functions. Transducer-calibrated-power-spectra yield the spectral-slope and midband-fit, parameters related to size and concentration, respectively. Since photoacoustic/ultrasound images are based on pressure waves generated from a spatial distribution of sources, we propose using photoacoustic-signal-envelope-statistics along with spectral parameters to characterize biological tissues.

In this study, gelatin-based-tissue-mimicking phantoms were constructed using black glass beads (Polysciences). Phantoms with 10-beads/resolution-volume (150, 180 and 250-um bead-diameter) were imaged using a 760-nm OPO and a 128-element/5-MHz-linear-array system (Ultrasonix). The 150-um phantom had 10, 12 and 15-beads/resolution-volume. Rayleigh-probability-density-functions were fit to the signal envelopes while computing the spectral-slope and midband-fit using a gold-film to remove system dependencies.

For all phantoms, the Rayleigh-probability-density-function was a good fit to the signal-envelope histograms, suggesting the presence fully-developed-speckle, likely due to the random bead positioning. The spectral-slope remained unchanged when the bead concentration increased by 1.5x; the midband-fit increased by 3-dB. Increasing the bead-diameter by 100-um, decreased the spectral-slope by 9x and increased the midband-fit by 7-dB, consistent with theoretical predictions. The results suggest that Rayleigh statistics and spectral parameters can potentially be used to monitor absorber size/concentration during photoacoustic imaging tissue characterization.

8943-85, Session 13

Modeling the shape of cylindrically focused transducers in three-dimensional optoacoustic tomography

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An efficient model-based reconstruction procedure in optoacoustics is based on discretizing the time-domain solution of the wave-equation Cauchy-problem. This leads to a matrix-vector-equation p=M?f. The model-matrix M establishes the relationship between deposited optical energy f and detected pressure waves p. Inversion is implemented by calculating the least-squares solution fsol=argminf ||p-M?f ||.

In principle, this model-based reconstruction assumes pressure waves being detected at single points in space. However, signals collected by actual transducers are the averaged pressure on its active surface which deviates from the point-transducer assumption. We analyze two different approaches to model the shape of cylindrically focused transducers.

First we approximated the transducer surface by a set of surface elements. Adding up model-matrices corresponding to each of the surface elements, one obtains a new matrix modeling the shape of the entire transducer.

The second approach is based on analytically calculating the spatial impulse response (SIR) of a line transducer. Thereby, cylindrically focused detectors can be approximated by n lines and its impulse response is estimated as the sum of n line impulse responses. Signals collected by the transducer are then modeled by temporal convolution of the SIR with the model-matrix assuming point transducers.

Both approaches yield new model-matrices for inversion incorporating the geometric properties of the transducer. The procedure suggested was tested in simulations and experiments with agar-phantoms containing microspheres and an ex-vivo mouse spleen. Typical artifacts

in optoacoustic tomography resulting from data collected by currently used ultrasonic transducers could be thoroughly corrected. Resolution of reconstructed images was improved in all spatial dimensions.

8943-86, Session 13

Acoustic-speed correction of photoacoustic tomography by ultrasonic computed tomography based on optical excitation of elements of a full-ring transducer array

Jun Xia, Chao Huang, Konstantin I. Maslov, Mark A. Anastasio, Lihong V. Wang, Washington Univ. in St. Louis (United States)

Photoacoustic computed tomography (PACT) is a hybrid technique that combines optical excitation and ultrasonic detection to provide high resolution images in deep tissues. In the image reconstruction, a constant speed of sound (SOS) is normally assumed. This assumption, however, is often not strictly satisfied in deep tissue imaging, due to acoustic heterogeneities within the object and between the object and coupling medium. If these heterogeneities are not accounted for, they will cause distortions and artifacts in the reconstructed images. In this paper, we incorporated ultrasonic computed tomography (USCT), which measures the SOS distribution within the object, into our full-ring array PACT system. Without the need for ultrasonic transmitting electronics, USCT was performed using the same laser beam as for PACT measurement. By scanning the laser beam on the array surface, we can sequentially fire different elements. As a first demonstration of the system, we studied the effect of acoustic heterogeneities on photoacoustic vascular imaging. We verified that constant SOS is a reasonable approximation when the SOS variation is small. When the variation is large, distortion will be observed in the periphery of the object, especially in the tangential direction.

8943-87, Session 13

Adaptive detection of molecular agents in multispectral optoacoustic tomography

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Detection of molecular agents and probes such as fluorescent proteins, dyes and photo-absorbing nanoparticles is a key application of optoacoustic (also termed photoacoustic) imaging. However, due to the high levels of background tissue absorption, multispectral measurements and spectral processing methods are required for accurately resolving the bio-distribution of such molecules within tissue. Multispectral optoacoustic tomography presents a very complex non-linear spectral un-mixing problem, where the perceived spectral features alter with tissue depth due to the wavelength dependent optical fluence attenuation. Moreover, various types of noise typically encountered in experimental reality further affect the measured spectra, forming a complex spectral clutter that is difficult to be modeled. In such conditions linear un-mixing approximations fail to provide an accurate performance, while non-linear inversion approaches based on light propagation models tend to be ill-posed, which limits their application in experimental tissue images. In this paper we describe a new solution to this problem that is based on the general adaptive sub-pixel detection framework. We introduce the main concepts of adaptive detection algorithms in the context of optoacoustic imaging specifying their field of application and propose modifications for enhancing their performance in molecular imaging applications. A quantitative comparison with linear un-mixing approaches on synthetic data-sets suggests an impressive performance enhancement with up to 5 times improved detection sensitivity, depending on the application. Corresponding performance is demonstrated on experimental multispectral optoacoustic images of mice in-vivo.

8943-88, Session 13

Fast tempo-spatial image reconstruction based on low-rank matrix estimation for dynamic photoacoustic computed tomography

Kun Wang, Jun Xia, Lihong V. Wang, Mark A. Anastasio, Washington Univ. in St. Louis (United States)

Photoacoustic computed tomography (PACT) is an emerging bio-imaging modality that exploits optical contrast and ultrasonic detection principles. In order to monitor dynamic physiological events in real time, a variety of PACT imaging systems have been developed that can acquire a frame of data in under a second. In conventional dynamic PACT, images are reconstructed by use of a static image reconstruction algorithm frame-by-frame. Frame-by-frame image reconstruction (FBFR) fails to exploit the strong correlations between data frames, thus sub-optimally mitigating image noise. Also, the FBFR can be computationally burdensome, particularly if advanced iterative image reconstruction algorithms are employed.

In this study, a low-rank matrix estimation-based image reconstruction in the singular system (LRME-SSR) of the data matrix is proposed. The LRME-SSR is based on a heuristic observation that the rank of the measured data matrix is much smaller than the number of frames for many PACT applications. When the low-rank assumption is valid, the required number of images to be reconstructed is reduced to the rank of the data matrix. Further, the low-rank prior is exploited to regularize data-domain de-noising by use of a low-rank matrix estimation algorithm. The performance of the LRME-SSR is compared with that of the conventional FBFR followed by image-domain filtering in both computer-simulated and experimentally-measured photoacoustic data. The results demonstrate that the LRME-SSR is not only computationally more efficient but also produces more accurate images than in the conventional FBFR followed by image-domain filtering.

8943-89, Session 13

Spectrum analysis of photoacoustic signals for tissue classification

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Quantitative ultrasound (QUS) estimates derived from power spectra of pulse-echo signals are sensitive to microstructure and potentially can differentiate among tissues. However, QUS estimates do not provide molecular specificity. We investigated the feasibility of obtaining quantitative photoacoustic (QPA) estimates for sensitivity to microstructure and chromophores for tissue classification. QPA methods were tested using gel-based phantoms containing uniformly dispersed, black polyethylene spheres (1E5 particles/ml) with nominal mean diameters of 23.5, 29.5, 42.0, and 58.0 microns. A pulsed, 532-nm laser excited the photoacoustic (PA) response. A single-element, 34-MHz transducer with a 12-mm focal length was raster scanned over the phantom to acquire 3D PA data. Normalized power spectra were generated from the PA signals within 2079, moving (50% overlap), 1-mm-cube regions-of-interest (ROIs) to provide three QPA estimates: spectral slope (SS), spectral intercept (SI), and effective absorber size (EAS). SS and SI were computed using a linear-regression approximation to the normalized spectrum in the -6-dB band. EAS was computed by fitting the normalized spectrum in the -20-dB band to the multi-sphere analytical solution. All estimates were correlated with the size of particles dispersed in the phantoms. SS decreased while SI increased with an increase in particle size. EAS was correlated with nominal particle diameter, but

particles aggregation and the finite bandwidth of the PAI system resulted in outliers. SS, SI, and EAS for the 23.5-micron-phantom were -0.14+/--0.04 dB/MHz, 4.8+/-1.3 dB, and 25.4+/-6.3 microns, respectively; the corresponding values for the 58.0-micron-phantom were -0.47+/--0.03 dB/MHz, 15.6+/-0.9 dB, and 82.7+/-0.9 microns.

8943-90, Session 13

Spatial over-sampling and its influence on spatial resolution for photoacoustic tomography with finite sized detectors

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Detector arrays enable parallel detection for faster photoacoustic imaging than by moving a single detector, but the detector spacing for arrays cannot be smaller than the size of an array element. Spatial over-sampling is scanning with a step-size smaller than the size of the detector element and is possible only for a moving single detector. For a detector with finite sized surface the measured acoustic signal is a spatial average of the pressure field over the detector surface. If the reconstruction is performed assuming point-like detection over-sampling brings no advantage as e.g. for spherical or cylindrical detection surfaces the blurring caused by a finite detector size is proportional to the distance from the rotation center and is equal to the detector size at the detection surface.

Iterative reconstruction algorithms or inverting directly the imaging matrix can take the finite size of real detectors directly into account, but the numerical effort is significantly higher compared to direct algorithms assuming point-like detection. Another reconstruction with less numerical effort is to use a direct algorithm and run a deconvolution algorithm for deblurring afterwards. For such reconstruction methods spatial over-sampling makes sense because it reduces the blurring significantly.

The effect of step size on the reconstructed image is systematically examined using simulated and experimental data. Experimental data are obtained on a plastisol cylinder with thin holes filled with an absorbing liquid. Data acquisition is done by utilization of piezoelectric detectors of various size and shape which are rotated around the plastisol cylinder.

8943-91, Session 14

Blood flow imaging using photoacoustic computed tomography

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High-resolution blood flow imaging in deep tissue offers valuable functional information for diagnosing and assessing many diseases. Doppler ultrasound is a longstanding technology that achieves high-resolution non-invasive flow imaging at depths. However, Doppler ultrasound suffers from poor sensitivity to blood, so it cannot measure slow flow in deep tissue. Photoacoustic (PA) tomography has recently been recognized as a promising technique for blood flow imaging. In shallow tissue, an optical beam can be effectively focused, allowing for sensing cellular-level features and then readily computing the flow speed. However, in deep tissue, fine features in blood cannot be well resolved. Thus PA blood flow imaging in deep tissue remains a challenge.

We developed a blood flow imaging method based on photoacoustic computed tomography, which allows for slow blood flow imaging in deep tissue. In this method, modulated ultrasound is focused into the flowing medium to generate confined heat sources. PA computed tomography is then utilized to image the heat propagation in the fluid. PA imaging of blood provides higher contrast than ultrasound imaging. Thermal waves propagate with the flow and are directly visualized in pseudo-color by photoacoustic computed tomography. The Doppler shift is employed to calculate the flow speed. This method requires only acoustic and optical absorption, and thus is applicable to continuous fluid. A blood flow speed as low as 0.24mm?s-1 was successfully measured. Deep blood flow imaging was experimentally demonstrated under 5-mm-thick chicken breast tissue.

8943-92, Session 14

Nonlinear photoacoustic spectroscopy of oxygenated and deoxygenated hemoglobin

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As intensity increases in photoacoustic microscopy (PAM), saturation of the optical absorption and the temperature dependence of thermal expansion result in a nonlinear dependence of the photoacoustic (PA) signal on the excitation pulse fluence. In the presence of nonlinearity, quantitative photoacoustic measurements at different wavelengths require a detailed analysis of the intensity-dependent PA signal. Laser spectroscopy of oxygenated blood based on thermal nonlinearity was previously reported. However, there have been no studies of the wavelength- and concentration-dependent effects of optical saturation and thermal nonlinearity on the PA signals of either oxygenated or deoxygenated hemoglobin. Here, we use a photoacoustic spectrometer with a flat-top beam illumination, which effectively reduces uncertainty in our measurements arising from inhomogeneous spatial distribution of the optical fluence. In oxygenated whole blood, we show that the wavelength dependence of the nonlinear PA spectrum is significantly larger than in deoxygenated whole blood. Moreover, we show how the nonlinear PA spectrum of oxygenated lysed blood is affected by different concentrations of the hemoglobin molecules. Careful selection of wavelengths can minimize these nonlinear effects and significantly improve accuracy of quantitative functional PAM.

8943-93, Session 14

Photoacoustic imaging of advanced renal cell carcinoma (RCC) tumor models for evaluating anti-angiogenic therapy efficacy

Peng Shao, David W. Chapman, Ronald B. Moore, Roger J. Zemp, Univ. of Alberta (Canada)

In this paper we present photoaocusitc imaging of the xenogeneic orthotopic human renal cell carcinoma (RCC) tumor model for evaluating therapy efficacy of the tyrosine kinase inhibitors (TKIs). Currently, treatments for advanced RCC have not been very successful. This has led to the development and investigation of a new group of drugs called tyrosine kinase inhibitors (TKIs). TKI's disrupt blood vessel formation and starve the tumor of its blood supply. Recently a subcutaneous RCC tumor model has been successfully established in the mouse ear. A serial study was conducted with this model to evaluate the therapy efficacy by visualizing blood vessel angiogenesis. The vasculature within and surrounding the tumor model in Balb c/nu-nu was imaged and regression or progression of angiogenesis was longitudinally observed with and without TKI treatment. Vasculature density, blood flow rate and oxygen saturation of target tumor areas were acquired from both the treatment and control group. Experiment results demonstrated capability of photoacoustic microscopy in functional imaging of cancer angiogenesis and verified feasibility of the TKIs treatment in reducing tumor growth.

8943-94, Session 14

Resting-state functional connectivity imaging of the mouse brain using photoacoustic tomography

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Resting-state functional connectivity (RSFC) imaging is an emerging neuroimaging approach that aims to identify spontaneous cerebral hemodynamic fluctuations and their associated functional connections. Clinical studies have demonstrated that RSFC is altered in brain disorders such as stroke, Alzheimer's, autism, and epilepsy. However, conventional neuroimaging modalities cannot easily be applied to mice, the most widely used model species for human brain disease studies. For instance, functional magnetic resonance imaging (fMRI) of mice requires a very high magnetic field to obtain a sufficient signal-to-noise ratio and spatial resolution. Functional connectivity mapping with optical intrinsic signal imaging (fcOIS) is an alternative method. Due to the diffusion of light in tissue, the spatial resolution of fcOIS is limited, and experiments have been performed using an exposed skull preparation. In this study, we show for the first time, the use of photoacoustic computed tomography (PACT) to noninvasively image resting-state functional connectivity in the mouse brain, with a large field of view and a high spatial resolution. Bilateral correlations were observed in eight regions, as well as several subregions. These findings agreed well with the Paxinos mouse brain atlas. Fore- and hind-paw stimulation experiments were then performed to confirm the locations of subregions in the somatosensory cortex. By subjecting the mouse to alternating normoxic and hypoxic conditions, strong and weak functional connectivities were observed, respectively. Moreover, functional connectivity was investigated in transgenic Alzheimer's mouse models, showing disruption in the connectivities. These studies show that PACT is a promising, non-invasive modality for small-animal functional brain imaging.

8943-95, Session 14

Photoacoustic measurement of nonradiative relaxation time and its application for in vivo measurements of blood oxygenation

Konstantin I. Maslov, Junjie Yao, Lihong V. Wang, Washington Univ. in St. Louis (United States)

In this work, using an optical resolution confocal photoacoustic microscope we modified the pulse probe technique to measure nonradiative relaxation times. Laser pulses of 3-ps duration were split and recombined twice. Time delays were introduced by varying the beams' path lengths. By blocking one of the beams, it was possible to deliver either a single pulse or two or four pulses delayed by a fixed time; the delay can be anywhere between 3 ps and 100 ps. Due to the very short laser pulse width, optical absorption everywhere within an acoustic voxel was highly saturated. Correspondingly, the absorbed laser energy and hence the photoacoustic signal depended only on the concentration of absorbers and their nonradiative relaxation times. Changes of laser fluence due to medium scattering and absorption did not influence the results. The technique was used for imaging blood oxygenation in small vessels, exploiting the large difference in relaxation time between oxyhemoglobin (~3 ps) and deoxyhemoglobin (~30 ps). For a single laser pulse, the photoacoustic signals from both states of hemoglobin are of similar amplitude. However, adding one or three 3 ps delayed pulses proportionally increases the photoacoustic signal from deoxyhemoglobin, while the signal from oxyhemoglobin remains unaffected.

8943-96, Session 14

Cancellous bone tissue imaging by photoacoustics and ultrasound modalities

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Cancellous bone consists of three-dimensional networks of plate and rod like trabeculae which generate a very complicated inhomogeneous and anisotropic porous medium. Imaging of cancellous bone with different modalities can reveal various features of bone tissue. Each modality induced by specific physical properties of tissue is sensitive to specific characteristics of bone. Investigating and understanding the sensitivity of each technique can help provide a viable technique for bone tissue monitoring.

In this study, we used ultrasound (US) and photoacoustic (PA) imaging modalities to characterize trabecular bone. The detector for both modalities was a 2.2 MHz transducer with lateral resolution of ~1 mm at focal point. With lateral resolution much lower than the size of trabeculae, the images generated spatially averaged contrast based on mechanical and optical properties in the focal area. PA signals were induced with an 800 nm CW laser that could generate high penetration depth. Depth resolved images were obtained by time-gating the signal of both modalities. Those images were compared with micro-computed tomography (?CT) images as gold standard, filtered to generate similar spatial resolution. The comparison revealed good correlations between PA and US modalities with the mineral volume fraction of bone tissue. Various features and properties of these modalities such as detectable depth, resolution and sensitivity will be discussed.

8943-97, Session 14

In vivo quantification of retinal oxygen metabolic rate in rodent

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Diabetic retinopathy (DR) is a leading eye disease; without immediate medical intervention at the stage of non-proliferative DR, it will progress into proliferative DR which causes vision blur and even vision loss. Yet, current detection methods can merely identify proliferative DR, preventing early DR treatment. The very early symptoms of non-proliferative DR involve retinal tissue hypoxia and retinal blood flow variation, indicating change of retinal oxygen metabolic rate (MRO2); thus detecting retinal MRO2 is important for early diagnosis and treatment of DR. Recently developed photoacoustic technique can precisely measuring blood oxygen saturation due to its capability to directly access of blood optical absorption. Here, we demonstrate in vivo measurement of retinal MRO2 in rat using integrated photoacoustic ophthalmology (PAOM) and spectral-domain optical coherence tomography (SD-OCT). We used multi-wavelength (570,578,588 nm) PAOM to measure oxygen saturation and vessel size of major retinal arteries and veins. The corresponding flow velocity within each imaged major vessel was measured by Doppler SD-OCT. The measured mean arterial and venous oxygen saturations were 93.0±3.5% and 77.3±9.1%, respectively; the measured mean arterial and venous flows were 2.14±0.59 μl/min and 2.57±0.57 μl/min, respectively. The estimated MRO2 in a normal adult rat was 22.60±37.32 ng/min.

8943-4, Session 15

Dual modality optoacoustic - laser ultrasound endoscopy system

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There is a pressing need for versatile and effective instrumentation capable of detecting esophageal and colorectal cancer in its early stages. Ultrasound imaging has been proven effective in identifying and staging relatively advanced tumors in esophageal or colon wall lining. Adding the optoacoustic imaging modality may prove beneficial in detecting early stage tumors, and precancerous tissue conditions such as Barrett's esophagus. Here we present a dual-modality optoacoustic - laser ultrasound (OA-LUS) endoscopy system with enhanced imaging capabilities. The system consists of a rotating 90off-axis parabolic reflector which is acoustically coupled to a flat 16-element ultrasound array. The ultrasound detector contains a central opening to accommodate an optical fiber for light delivery. A parabolic mirror with a diameter of 12.7 mm and a parent focal length of 15 mm reflects laser pulses towards a sample at 90° with respect to the axis of rotation and reflects incoming optically generated ultrasound signals towards a detector. The laser ultrasound imaging modality is enabled by placing an optically absorbing polymeric membrane in the path of laser light to generate broadband and non-reverberating ultrasound wave packets propagating towards the sample. Reflected ultrasound signals pass through the thin layer without significant distortions, and are reflected towards the ultrasound array. Performance of the dual modality OA-LUS system in terms of resolution, imaging depth, and the rate of data acquisition will be presented and discussed.

8943-99, Session 15

Optoacoustic microscopy using probe beam deflection technique

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Optoacoustic microscopy (OAM) is an emerging technology combining the beneficial features of optics and ultrasound, to form a hybrid imaging technique capable of multiscale, high-resolution imaging. In the last decade, conventional optoacoustic imaging has been developed into a viable tomographic (macro) imaging technique, but it has been limited by its resolution in the arena of microscopy. Current detection methods used in optoacoustic imaging are Ultrasound-Resolution Optoacoustic Microscopy and Optical-Resolution Optoacoustic Microscopy. These methods have physical constraints that limit them in either axial or spatial resolution (or both). Here we introduce an innovative, all-optical method for detecting optoacoustic signals: the probe beam deflection technique (PBDT). The PBDT overcomes the limitations of conventional ultrasound transducers (e.g. piezoelectric), thus far adapted for optoacoustic imaging, primarily by increasing the working distance between objective lens and sample, allowing the use of high numerical aperture objectives. It also provides a non-contact and non-destructive method that is less sensitive to background noise. In PBDT, the acoustic pressure wave is detected indirectly as it propagates through a detection chamber and interacts with the probe beam. The PBDT sensitivity is controllable by parameters such as probe beam power, spot size, interaction length, probe beam wavelength, photodiode sensitivity and proximity to sample. The basic setup of PBDT shows a high sensitivity competitive with commercial conventional transducers. We report first images of different biological tissues obtained using this technique. Images of test samples showed initial spatial resolution of 6um only limited by the step size of the mechanical scanning stage.

8943-100, Session 15

In vivo ultrasound and photoacoustic monitoring of burn skin regeneration promoted by adipose-derived stem cell treatment

Seung Yun Nam, Eunna Chung, Laura J. Suggs, Stanislav Y. Emelianov, The Univ. of Texas at Austin (United States)

Burn injuries are a serious public health problem and cause a huge and intolerable burden of death and disability. Recently, stem cell therapy has been investigated in the treatment of burns due to the excellent regenerative potential of stem cells. However, in order to control therapeutic efficiency and to prevent side effects, including inflammation and hypertrophic scar formation, regenerative procedures of the burned skin need to be monitored by quantitative measurement of critical factors such as vascular changes and tissue mechanical properties. Towards this end, ultrasound and photoacoustic imaging is a promising technique because it can synergistically provide both functional and mechanical information about the tissue in vivo. In this study, we demonstrated in vivo ultrasound and photoacoustic monitoring of burn skin regeneration promoted by stem cell treatment. The rat dorsal skin was burned using a heated brass disk connected to a thermocouple probe. For the treatment of the burned skin, adipose-derived stem cells incorporated with PEGylated fibrin gel were placed at the burned region after excision of the injured tissue. Before and after the burn surgery, photoacoustic imaging was performed to compare the directional and dimensional changes of microvasculature around a burn injury region. Also, tissue mechanical properties were noninvasively measured by ultrasound imaging. This study demonstrates that the ultrasound and photoacoustic imaging technique can be successfully utilized to track burn tissue regeneration procedures in vivo.

8943-101, Session 15

Experimental investigation into the suitability of classes of exogenous contrast agents for thermoacoustic imaging

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Thermoacoustic imaging lacks the spectroscopic capabilities and tissue specificity of photoacoustic imaging because the main source of endogenous contrast is the water and ionic content in tissue. So while the contrast between high water content tissue and adipose dominated tissue might be as much as an order of magnitude, it can be as little as 10% between two high water content tissues. Therefore, the use of exogenous contrast agents to selectively increase the contrast of one high water content tissue relative to another, with or without targeting, is very appealing. An example of such an application is the imaging of vasculature in which an intravenous injection of contrast agents increases the contrast of blood relative to the vessel walls, both of which have high water content.

While a number of contrast agents used in photoacoustic and MRI, have been investigated for thermoacoustic applications, a detailed characterisation of their dielectric and magnetic properties has never been done, leading to speculation as to the source(s) of increase contrast observed. To this end, we take a fundamental look at the sources of contrast found in several contrast agents including iron oxide particles, carbon nanotubes, three different gadolinium based contrast agents and some novel salt complexes.

By characterising the dielectric and magnetic properties of the contrast agents at 3GHz, using a cavity resonator, as well as measuring the DC conductivity, we show that the dipole rotational loss in most cases contributed more to the total measured conductivity, than the ionic conductivity. From single point thermoacoustic measurements as well as 3D electromagnetic simulations, we show that the increase in the thermoacoustic signal amplitude measured in solutions of contrast agents is not due solely to the measured changes in conductivity but also the changes in real part of the complex permittivity as well increases which occurs in the Gruneisen parameter of the solutions.

8943-102, Session 15

Handheld optical fiber parallel acoustic delay line (PADL) probe for photoacoustic tomography

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In current photoacoustic tomography (PAT), I-D or 2-D ultrasound arrays and multi-channel data acquisition (DAQ) electronics are used to detect the photoacoustic signals simultaneously for “real-time” image construction. However, as the number of transducer elements and DAQ channels increase, the construction and operation of the ultrasound receiving system will become complex and costly. This situation can be addressed by using parallel acoustic delay lines (PADLs) to create true time delays in multiple PA signal channels. The time-delayed PA signals will reach the ultrasound transducer at different times and therefore can be received by one single-element transducer without mixing with each other.

In this paper, we report the development of the first miniaturized PADL probe suitable for handheld operations. Fused-silica optical fibers with low acoustic attenuation were used to construct the 16 PADLs with specific time delays. The handheld probe structure was fabricated using precision laser-micromachining process to provide robust mechanical support and accurate alignment of the PADLs with minimal acoustic distortion and inter-channel coupling. The 16 optical-fiber PADLs were arranged to form one input port and two output ports. Photoacoustic imaging of a black-ink target embedded in an optically-scattering phantom was successfully conducted using the handheld PADL probe with two single-element transducers and two DAQ channels (equal to a channel reduction ratio of 8:1). Our results show that the PADL technique and the handheld probe could provide a promising solution for real-time PAT with significantly reduced complexity and cost of the ultrasound receiver system.

8943-103, Session 15

In vivo ultrasound-guided photoacoustic imaging of ischemic tissue regeneration enhanced by mesenchymal stem cells labeled with nanoparticles

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Recently, stem cell therapy has shown promise as an alternative treatment for ischemic diseases because stem cells can improve tissue function via differentiation and paracrine effects. During the therapeutic regeneration process, it is important to track stem cell behaviors and monitor tissue regeneration indicators, such as neovascularization and macrophage infiltration. Ultrasound-guided photoacoustic imaging is an ideal candidate to verify ischemic tissue regeneration due to minimal invasiveness with high spatial resolution and great sensitivity/selectivity with various contrast agents, including hemoglobin and metallic nanoparticles. In this study, ischemic tissue regeneration in the rat hindlimb muscle was monitored in vivo using ultrasound-guided molecular photoacoustic imaging. Specifically, to promote vascularization and tissue regeneration, mesenchymal stem cells (MSCs) labeled with silica coated gold nanorods were injected within PEGylated fibrin gel into the ischemic hindlimb muscle of a rat following femoral artery ligation. In addition, PEGylated gold nanospheres were intramuscularly injected to preferentially label infiltrated macrophages. Owing to unique and stable optical properties of hemoglobin and the labeled MSCs and macrophages, neovasculature and macrophage infiltration, as well as MSC migration, could be selectively tracked over a one week time period. The results indicate that ultrasound-guided photoacoustic imaging can be a promising technique for noninvasive longitudinal monitoring of ischemic tissue regeneration enhanced by stem cell implantation.

8943-104, Session 15

All-optical photoacoustic microscopy (AOPAM) system for remote characterization of biological tissues

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Conventional photoacoustic microscopy (PAM) employs light pulses to produce a photoacoustic (PA) effect and detects the resulting acoustic waves using an ultrasound transducer acoustically coupled to the target. The resolution of conventional PAM is limited by the sensitivity and bandwidth of the ultrasound transducer. We investigated a versatile, all-optical PAM system for characterizing ex vivo and ultimately in vivo tissues. The system employs non-contact interferometric detection of PA signals that overcomes limitations of conventional PAM. A 532-nm pump laser with a pulse duration of 5 ns excites the PA effect in tissue. Resulting acoustic waves produce surface displacements that are sensed using a 532-nm continuous-wave (CW) probe laser in a Michelson interferometer with a 1-GHz bandwidth. The pump and probe beams are coaxially focused using a 50X objective giving a diffraction-limited spot size of 0.48 μm. The phase-encoded probe beam is demodulated using homodyne methods. The detected time-domain signal is time reversed using k-space wave-propagation methods to produce a spatial distribution of PA sources in the target tissue. A minimum surface-displacement sensitivity of 0.19 pm was measured. PA-induced surface displacements are very small; therefore, they impose stringent detection requirements and determine the feasibility of implementing an all-optical PAM in biomedical applications. 3D PA images of ex vivo porcine retina specimens were generated successfully. We believe the all-optical PAM system is well suited for assessing retinal diseases and other near-surface biomedical applications such as sectionless histology and evaluation of skin burns and pressure or friction ulcers.

8943-105, Session 16

Optical generation of narrowband high frequency ultrasound

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High frequency ultrasound can be generated when a thin film is irradiated with an optical pulse. Spectrum of the ultrasound signal is primarily determined by the laser pulse width and the film thickness. Traditionally, a

ns pulsed laser and a single layer film are used, resulting in an ultrasound signal with a very wide fractional bandwidth. In some applications, nonetheless, it is desirable to have the ability to reduce bandwidth of the ultrasound signal. In this study, we propose a multilayer film structure to generate narrowband ultrasound. Our multilayer structure consists of several light-absorbing layers and light-transmittance layers. Specifically, graphite-polymer mixtures and pure PDMS are used as the light-absorbing material and the light-transmittance material, respectively. The graphite-polymer mixture is uniformly spin coated with pure PDMS layer by layer. The amplitude of each thermoelastic ultrasound signal is tunable by adjusting the optical absorption coefficient of light-absorbing layers. In addition, the delay between thermoelastic ultrasound signals generated by light-absorbing layers can be adjusted by changing thicknesses of light-transmittance layers. To measure waveform of the generated narrowband high frequency ultrasound signal, a hydrophone (MHA9-150, Force Technology, Denmark) is used. In one example, the generated high frequency narrowband ultrasound signal has a center frequency of 18.4MHz and 32.6% fractional bandwidth using the proposed multilayer structure. On the other hand, the single layer structure produces a center frequency of 20.2MHz and 125.7% fractional bandwidth. Feasibility of the proposed design is therefore verified.

8943-106, Session 16

Improving focusing efficiency in scattering media: spectrally filtered photoacoustic-guided wavefront shaping

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Focusing light to the micron scale is crucial for biomedical applications such as optical microscopy and laser micro-surgery. However, light scattering limits effective focusing to shallow depths of a few hundred micrometers. Wavefront shaping appears in the last few years as a powerful tool to restore optical focusing of a beam after propagation through a scattering medium. However the main challenge for imaging application is to achieve focusing inside scattering media. Photoacoustic effect has already been proposed to monitor the light intensity at the target location, and efficient optimization of the photoacoustic response of micrometer-sized absorbing particles has been demonstrated by Kong et al. in 2011 (Opt. Lett. 36, 2053-2055).

However, the frame of this demonstration was somewhat restrictive, because of the small size of these absorbers compared to the size of the acoustic focus. As hemoglobin is the main endogenous absorber in, one will more likely deal with extended absorbers (i.e. larger than the acoustic focus at least in one dimension), such as blood vessels. Since the increase in the photoacoustic signal is inversely proportional to the number of optical modes contained in the probed region of the absorber, the optimization of the broadband photoacoustic response (coming from a wide acoustic focal zone) of these extended absorber will be poorly efficient. We experimentally demonstrate that the optimization of the high frequency content of the photoacoustic signal is a convenient way to overcome this limitation. We confirm with numerical simulations that this high frequency band optimization leads to tighter optical foci.

8943-107, Session 16

Optical-fiber based all-optical 3D photoacoustic imaging system

Yusuke Miida, Yuji Matsuura, Tohoku Univ. (Japan)

An all-optical 3D photoacoustic imaging probe that consists of an optical fiber probe for ultrasound detection and a bundle of hollow optical fibers for excitation of photoacoustic waves was developed. We first fabricated an optical fiber probe for ultrasound detection. The probe consists of a single-mode optical fiber with a thin polymer film attached to the output end surface for detection of acoustic waves. Fiber-coupled optical components for optical communication, such as a DFB laser diode and optical circulator, were used to construct a stable and low-cost Fabry Perot interferometer. As a result of evaluating its operating characteristics, we confirmed that the probe had almost omnidirectional reception and a high SNR that is equivalent to those of common PVDF hydrophones. The photoacoustic fiber probe also successfully took B-mode images of the blood vessel phantoms when combined with a single optical fiber for excitation of photoacoustic waves. Then we fabricated a 3D photoacoustic imaging system that consists of a photoacoustic fiber probe and a bundle of hollow optical fibers. Owing to the extremely small NA of the hollow fiber, an image resolution that is the same as the diameter of the hollow optical fiber was obtained. In addition, without any scanning mechanism at the distal end, 3D imaging can be performed by subsequently exciting the hollow fibers at the input end of the hollow fiber bundle. After some image processing, a 3D image of the blood vessel phantom with a resolution of around 0.3 mm was successfully reconstructed.

8943-108, Session 16

Non-contact photoacoustic tomography with a laser Doppler vibrometer

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Most concurrent photoacoustic tomography (PAT) systems are based on traditional ultrasound measurement regime, which requires the contact or acoustic coupling material between the biological tissue and the ultrasound transducer. This study investigates the feasibility of non-contact measurement of photacoustic signals generated inside biomedical tissues by observing the vibrations at the surface of the tissues with a commercial laser Doppler vibrometer. The vibrometer with 0-2MHz measurement bandwidth and 5 MHz sampling frequency was integrated to a conventional rotational PAT data acquisition system. The data acquisition of the vibrometer was synchronized to the laser illumination from an Nd:YAG laser with output at 532nm. The laser energy was tuned to 20mJ per square centimeter. The PA signals were acquired at 120 angular locations uniformly distributed around the scanned objects. The frequency response of the measurement system was first calibrated. 2-inch-diamater cylindrical phantoms containing small rubber plates and biological tissues were afterwards imaged. The phantoms were made from 5% intralipid solution in 10% porcine gelatin to simulate the light scattering in biological tissue and to backscatter the measurement laser from the vibrometer. Time-domain backprojection method was used for the image reconstruction. The results validated our hypothesis that this non-contact PAT system can identify the inclusions with optical contrasts in the phantoms.

8943-109, Session 16

Photoacoustic imaging of a near-infrared fluorescent marker based on excited state lifetime modulation

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Photoacoustic imaging has been used to determine the spatial distribution of fluorophores, such as exogenous dyes and genetically expressed proteins, from images acquired in phantoms and in vivo. Most methods involve the acquisition of multiwavelength images and rely on differences in the absorption spectra of the tissue chromophores to estimate their spatial distribution and abundance using spectral decomposition techniques, such as model based inversion schemes. However, the inversion of 3-D images can be computationally expensive. Experimental approaches to localising contrast agents may therefore be useful, especially if quantification is not essential. This work aims to develop a method for determining the spatial distribution of a fluorescent cell marker (Atto680) from images acquired using dual wavelength excitation. The wavelengths (680nm, 740nm) coincide with the absorption and emission spectrum of the fluorophore, respectively. The contrast mechanism relies on modulating the excited state lifetime of the fluorophore by varying the time delay between the pulses. This changes the absorption of the fluorophore, and hence the photoacoustic signal amplitude. Since this is not observed in endogenous chromophores, the background may be removed by subtracting two images acquired with and without pulse delay. To characterize the fluorophore, the wavelength dependence of the signal amplitude is measured in a cuvette as a function of pulse delay, concentration, and fluence. The spatial distribution of the fluorophore is determined from images acquired in realistic tissue phantoms. This method may be suitable for in vivo applications, such as imaging of exogenous or genetically expressed fluorescent cell markers.

8944-1, Session 1

Controlling host's immunoregulatory cell populations secures the efficacy of photodynamic therapy-generated cancer vaccines *(Invited Paper)*

Mladen Korbelik, Judit Banath, The BC Cancer Agency Research Ctr. (Canada)

Discovery that photodynamic therapy (PDT)-treated tumor cells or their lysates can act as a vaccine for autologous mouse tumors has prompted the development of PDT-generated cancer vaccines. We have been engaged in ongoing efforts to optimize the activity of these vaccines, including PDT treatment and post-treatment parameters, vaccine cell numbers, and inoculation sites. It has become clear that PDT vaccine protocol has the potential to produce a very strong immune response against the vaccinated tumor. Nonetheless, the evidence also emerged that inducing such potent anti-tumor immune reaction is not sufficient to guarantee an effective therapeutic outcome. It will be shown that the underlying problem lies in the activity of immunoregulatory cell populations. For instance, the numbers of regulatory T cells was found to increase substantially in PDT-vaccinated tumors. However, treatment of host mice with low-dose cyclophosphamide (50 mg/kg) proved effective in reducing dramatically the induction of tumor invasion by these immuno-suppressing cells after PDT vaccine treatment, which was seen also as the reduction in the incidence of CD25+Foxp3+ cells among helper T lymphocytes. Another immunoregulatory cell population with a role in dampening immune response in some tumor models is comprised of myeloid-derived suppressor cells (MDSC). Since experience in clinic has shown that targeting these cells can enhance immune intervention in some cancer patients, strategies will be discussed on exploiting this approach with PDT-generated cancer vaccines. In conclusion, for securing the therapeutic efficacy of PDT-generated cancer vaccines as well as other immunotherapy protocols it is compulsory to characterize the associated tumor type specific immunoregulatory activity and incorporate appropriate measures for contravening their function.

8944-2, Session 1

Photodynamic therapy for melanoma: efficacy and immunologic effects *(Invited Paper)*

Pinar Avci, Gaurav K. Gupta, Masayoshi Kawakubo, Michael R. Hamblin, Wellman Ctr. for Photomedicine (United States)

Malignant melanoma is one of the fastest growing cancers and if it cannot be completely surgically removed the prognosis is bleak. Melanomas are known to be particularly resistant to both chemotherapy and radiotherapy. Various types of immunotherapy have however been investigated with mixed reports of success. Photodynamic therapy (PDT) has also been tested against melanoma, again with mixed effects as the melanin pigment is thought to act as both an optical shield and as an antioxidant. We have been investigating PDT against malignant melanoma in mouse models. We have compared B16F10 melanoma syngenic to C47BL/6 mice and S91 Cloudman melanoma syngenic to DBA2 mice. We have tested the hypothesis that S91 will respond better than B16 because of higher expression of immunocritical molecules such as MHC-1, tyrosinase, tyrosinase related protein-2 gp100 , and intercellular adhesion molecule-1. Some of these molecules can act as tumor rejection antigens that can be recognized by antigen-specific cytotoxic CD8 T cells that have been stimulated by PDT. Moreover it is possible that DBA2 mice are intrinsically better able to mount an anti-tumor immune response than C57BL/6 mice.

8944-3, Session 1

Glycated chitosan as a vaccine adjuvant

Pinar Avci, Gaurav K. Gupta, Masayoshi Kawakubo, Ji Wang, Wellman Ctr. for Photomedicine (United States); Wei R. Chen, Univ. Central Oklahoma (United States); Tomas Hode, Immunophotonics, Inc. (United States); Mei X. Wu, Michael R. Hamblin, Wellman Ctr. for Photomedicine (United States)

Vaccine adjuvants enhance and direct the adaptive immune response to vaccine antigens. This immune response is mediated by two main types of lymphocytes, B and T cells. Upon activation by cytokines, B cells differentiate into memory B cells (long-lived antigen-specific B cells) or plasma cells (effector B cells that secrete large quantities of antibodies). Most antigens activate B cells using activated T helper (Th) cells, primarily Th1 and Th2 cells. The magnitude and type of Th response to a vaccine can be greatly modulated through the use of adjuvants. For almost 80 years, aluminum salts (referred to as 'alum') have been the only adjuvant in use in human vaccines. As our understanding of the mechanisms of 'immunogenicity' and 'adjuvancy' increases, new adjuvants and adjuvant formulations are being developed. Glycated chitosan (GC) is a derivative of chitosan produced by attaching galactose molecules to the chitosan molecules. GC was designed for immune stimulations in combination with phototherapies in the cancer treatment. Although the mechanism of action of GC as an immunostimulant is not completely clear at present, we hypothesize that it behaves as a calcium dependent (C-type) lectin and binds to specific receptors (CLRs) expressed on dendritic cells (DC) thus increasing antigen uptake and improving presentation. We have tested GC in combination with intradermal vaccination of mice using ovalbumin and influenze vaccine (Afluria, mainly H1N1 and H3N2 strains). GC increased the antibody titer (over vaccine alone) in two different strains of mice with both vaccines, and antibodies appeared earlier. IgG1 and IgG2a titers showed that GC induced an appropriate mixture of Th1 and Th2 responses.

8944-4, Session 1

In vitro therapeutic effect of PDT combined with VEGF-A gene therapy

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Sirtuins (SIRTs) are the mammalian homologues of the silent information regulator 2 (Sir2) in yeast, and SIRTs are Class III NAD+-dependent histone deacetylase (HDAC) requires NAD+ as a cofactor, affecting cell survival as well as apoptosis. Photodynamic therapy (PDT) is a novel therapeutic modality to treat cancer by using a photosensitizer which is activated by a light source to produce reactive oxygen species and mediates oxygen-independent hypoxic conditions to tumor. Another emerging treatment to cure cancer is the use of interference RNA (e.g. siRNA) delivered by a sustainable drug delivery vehicle such as the lipid-calcium-phosphate nanoparticles (LCP), a liposome based drug delivery vehicle for nucleic acids. Cell viability decreased at the highest concentration of transfected SIRT1 or selected siRNA. Combined therapy of PDT and SIRT1 siRNA encapsulated LCP shows better therapeutic effect as tumor size decreased after treatment. The results suggest that PDT combined with targeted gene therapy has a potential mean to achieve better therapeutic outcome as compared to PDT and gene therapy alone.

8944-5, Session 2

In situ photoimmunotherapy and t-cell simulations in melanoma treatment *(Invited Paper)*

Wei R. Chen, Univ. of Central Oklahoma (United States); Mark F. Naylor, Dermatology Associates of San Antonio (United States); Robert E. Nordquist, Wound Healing of Oklahoma (United States)

T-cell stimulators such as anti-CTLA-4 antibodies or the newer (and still experimental) PD-1 and PD-L1 antibodies enhance immunologic responses to chemotherapy-resistant solid tumors, such as melanoma, advanced breast cancer, ovarian cancer and pancreatic cancer. The efficacy of these new immunotherapy agents can in theory be enhanced substantially by therapies that stimulate new immunologic responses against the tumor.

In Situ Photoimmunotherapy (ISPI) with imiquimod and InCVAX are techniques that produce useful responses in patients with advanced melanoma, the prototypical chemotherapy resistant solid tumor. The mechanism of action of these therapies is thought to be immunological, including the development of new T-cell responses. We have therefore been combining ISPI using imiquimod and InCVAX treatment with the new T-cell stimulators (ipilimumab) in cases of stage IV melanoma. While still anecdotal, the use of novel combinations of immunologic therapies should provide improved responses for chemotherapy-resistant solid tumors (such as melanoma) than was previously possible.

8944-6, Session 2

Effects of laser immunotherapy on tumor microenvironment

Joseph T. Acquaviva III, Wei R. Chen, Melville B. Vaughan, Univ. of Central Oklahoma (United States); Hong Liu, The Univ. of Oklahoma (United States); Eric Howard, The Univ. of Oklahoma Health Sciences Ctr. (United States)

The microenvironments of tumors are involved in a complex and reciprocal dialog with surrounding cancer cells. Any novel treatment must consider the impact of the therapy on the microenvironment. Recently clinical trials with laser immunotherapy (LIT) have proven to effectively treat patients with late-stage metastatic breast cancer and melanoma. LIT is the synergistic combination of phototherapy (laser irradiation) and immunological stimulation. One prominent cell type found in tumor stromas are fibroblast cells. Fibroblast cells can secrete different growth factors and extracellular matrix modifying molecules. Additionally, fibroblast cells differentiate into myofibroblasts. The malignancy of a tumor can be associated with the expression of myofibroblasts and different growth factors as well as extracellular matrix modifying molecules secreted by fibroblasts and myofibroblasts. To elucidate the effect LIT has on the microenvironment of tumors, a collagen lattice assay with human fibroblast cells is utilized. The contraction of the lattice, the differentiation of fibroblasts, as well as the proliferation of fibroblasts and myofibroblasts at different wattages and concentration of immunostimulant will be determined. Additionally, RNA expression of treated fibroblast cells and myofibroblast cells will be observed.

8944-7, Session 2

Effects of cyclophosphamide on laser immunotherapy for the treatment of metastatic cancer

Cody F. Bahavar, Wei R. Chen, Univ. of Central Oklahoma (United States); Tomas Hode, Immunophotonics, Inc. (United States); Robert E. Nordquist, Univ. of Central Oklahoma (United States); Hong Liu, The Univ. of Oklahoma (United States)

Laser immunotherapy (LIT) is an innovative cancer modality that uses laser irradiation and immunological stimulation to treat late-stage, metastatic cancers. The current mode of operation in LIT is through interstitial laser irradiation. Although LIT is still in development, recent clinical trials have shown that it can be used to successfully treat patients with late-stage breast cancer and melanoma. Cyclophosphamide is a chemotherapy drug that suppresses T-regulatory cells. In this study, tumor-bearing rats will be treated by LIT using an 805-nm laser with a power of 1 to 3 W and various doses of cyclophosphamide. Glycated chitosan will be used as an immunological stimulant. The goal is to observe the effects of differing doses of cyclophosphamide in addition to LIT on the survival of the tumor-bearing rats.

8944-8, Session 2

Laser immunotherapy and the tumor-immune system interaction: a mathematical model and analysis

Sean M. Laverty, Bryan Dawkins, Wei R. Chen, Univ. of Central Oklahoma (United States)

We create, present, and analyze a mathematical model consisting of a system of differential equations that describes the population dynamics of tumor cells, immune cells, and other immune mediators in a host undergoing laser immunotherapy treatment against metastatic cancer. We outline characteristics of the immune system and treatment program under which long-term control or elimination of cancer is possible. First, we identify ranges for the rate and efficiency parameters of host immune system. Second, we explore the sensitivity of dynamics to details of the treatment program by varying the modeled effects of the laser treatment pulse (e.g., pulse length or strength) and immunoadjuvant. We pay particular attention to the targets of immunoadjuvants within the immune system and identify the strength of immunoadjuvant effects required to create an immune response capable of controlling or eradicating tumors and metastases. From these comparisons we also identify conditions under which laser immunotherapy is insufficient to eliminate the focal tumor or metastases. Where possible model predictions are compared to available laboratory and clinical trial data (e.g., cell counts, cell dynamics, and whole organism survival). In particular, we are interested in the long-term maintenance of anti-tumor immunity (humoral or long-lived cellular) and plan to extend the model, if necessary, by including anti-cancer antibody production which will allow us to study cancer-immune dynamics following surgical re-implantation of secondary tumors.

8944-9, Session 3

Improvement for in vivo flow cytometer detection in ear skin by optical clearing agents *(Invited Paper)*

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The in vivo flow cytometry (IVFC) has shown a great potential for detecting circulating tumor cells quantitatively in the bloodstream. IVFC is based on the confocal excitation and detection of fluorescently labeled cells in circulation. It has the capability to count fluorescently labeled circulating cells in live animals, which can be applied to study the mechanism that govern the early steps in tumor cell spreading through the body. However, like other optical methods, the detection depth of IVFC is limited, due to strong light scattering within tissues. Optical

clearing by immersion of tissues into optical clearing agents (OCAs) can improve light penetration depth in tissues and enhance optical imaging depth. Recently, we have developed an optical clearing agent, namely, ESOCA, which can make the ear skin more transparent, and also improve the contrast for laser speckle imaging. Here, we are comparing the signal intensity, peak numbers and detection depth in the IVFC measurements and investigating the efficacy of ESOCA for improving the IVFC detection.

8944-10, Session 3

In vivo ultrasensitive flow cytometry for theranostics of bacteremia *(Invited Paper)*

Ekaterina I. Galanzha, Univ. of Arkansas for Medical Sciences (United States)

The gold standard for the therapy of staphylococcal infections is antibiotics. However, the timeline from the discovery of new antibiotics to their application in clinics takes several years, while the generation time for bacteria is only a few hours, if not minutes. This disproportion leads to a well-established problem of multi-drug resistance, and as a result, to high mortality rates, especially from bacteremia (≤40%). We plan to overcome this challenge by the development a new approach of real-time molecular detection, counting and targeted eradication of bacteria in body fluids by the use of photoacoustic (PA) and photothermal (PT) flow cytometry and functionalized low-toxic nanoparticles. With the mouse model of staphylococcal bacteremia (Staphylococcus aureus), in vivo utility of this platform was demonstrated at a single bacterium level in the blood circulation with sensitivity down to 0.5 CFU/mL, and in associated tissues following bacteria extravasation. Compared to existing diagnostic approaches, our technical platform may offer many advantages such as: (1) ultra-high sensitivity (0.5 CFU/mL); (2) integration of multiplex PA molecular detection and targeted PT killing of circulating bacteria with real-time PA monitoring of therapeutic efficacy; (3) advanced assessment of cerebrospinal fluid; and (4) high spectral specificity based on distinct spectral properties of nanoparticles. Because some jnanoparticles were already approved for pilot studies on humans, and the clinical potential and safety of PA technology has been successfully demonstrated in pilot clinical trials, early advanced, laser-based diagnosis of bacteremia has the potential of translation to humans.

8944-11, Session 3

Multi-color intravital optical imaging of tumor immunotherapy *(Invited Paper)*

Zhihong Zhang, Shuhong Qi, Qingming Luo, Britton Chance Ctr. for Biomedical Photonics (China)

Recently there has been remarkable progress in immunotherapy of melanoma patients using the adoptive T cell therapy combined with depletion of regulatory T cells (Tregs). Tregs play important roles in immunosuppression in tumor environment. Cyclophosphamide (CY) is a conventional Chemotherapy drug for cancer and recent studies suggest that low dose of CY is able to deplete Tregs and increase the antitumor immune responses after adoptive T cell therapy. Although this combined treatment is effective, the underlying mechanism of the low dose CY increases the antitumor activity by adoptive T cell is still not well understood. In our study, we used adoptive T cell therapy combined with low dose CY to treat B16 melanoma in mice and a window chamber model to dynamically in vivo observe tumor infiltrating lymphocytes (TILs) migration and movement in the tumor microenvironment. Results show that significantly enhanced tumor suppression was observed in the combined treatment group compared with control groups. After low dose CY treatment (100 mg/kg), the number of Tregs was significant decreased in tumor microenvironment compared to untreated groups. Furthermore, the TILs infiltrated into tumor areas were significant increased in the combined treatment group and the intratumoral migration remained active by intravital imaging. We conclude that by intravital microscopy imaging, we confirmed that low dose CY could deplete Tregs in B16 melanoma environment, increase the number of TILs infiltration into tumor areas and improve their intratumoral migration. In this way, the combined treatment activated antitumor immune responses to successfully control the B16 melanoma in mice.

8944-12, Session 3

Assessment of vascular changes induced by immune reaction by using laser speckle imaging with multi-exposure time *(Invited Paper)*

Vyacheslav Kalchenko, Yuri Kuznetsov, Dina Preise, Weizmann Institute of Science (Israel); Igor V. Meglinski, Univ. of Otago (New Zealand); Alon Harmelin, Weizmann Institute of Science (Israel)

With the advances of biomedical technologies and introduction of new drugs and biologic materials in clinical practice the needs in evaluation and prediction of possible immune reactions, such as irritations, allergies and other are clearly recognized. Current methods to identify irritants and/or allergens rely on a panel of conventional tests such as mouse ear swelling test (MEST), guinea pig maximization test or local lymph node assay (LLNA). These tests, although accepted by medical community suffer from several drawbacks some of them being based on subjective human factor-dependent evaluation or utilizing radioactive materials. These examination approaches are also time and animal consuming. Therefore, new non-invasive cost effective diagnostic modalities in the field are urgently required. We present a laser speckle based imaging technique specially developed for visualization and non-invasive quantitative assessment of the immune-mediated tissue response manifested as the increasing vascular permeability. We demonstrate that irritant-induced acute vascular changes can be detected by the laser speckle imaging showing a potential of this method in investigating vascular events mediated by irritants and allergens, as well as for predicting irritation and/or allergenic potential of new materials.

8944-13, Session 3

Imaging marine virus CroV and its host Cafeteria roenbergensis with two-photon microscopy

Bin Cao, The Univ. of Texas at El Paso (United States) and The Univ. of Texas at El Paso (United States); Sayan Chakraborty, Wenqing Sun, University of Texas at El Paso (United States); Seyedmohammadali Aghvami, The Univ. of Texas at El Paso (United States); Matthias G Fischer, Max Planck Institute for Medical Research (Germany); Wei Qian, University of Texas at El Paso (United States); Chuan Xiao, Chun Qiang Li, The Univ. of Texas at El Paso (United States)

Life is dynamic and most microorganisms are highly movable owing to the existence of a flagellum. Cryo-electron microscopy (cryo-EM), in which the sample is kept under liquid nitrogen or liquid helium temperature has become a powerful tool to study biomolecular structures, and in particular the infectious process of viruses. However, if the biological event under study is very dynamic and moving fast, it is extremely difficult to use cryo-EM to capture the specific moment without searching impracticable number of samples. Optical fluorescence microscopy is an attractive tool for scientist to explore dynamic processes in living specimens at cellular and subcellular level. In this project we combine cryo-EM with two-photon microscopy to study the infection process of marine zooplankton, Cafeteria roenbergensis (Cro), by CroV, a giant Nucleocytoplasmic large DNA and nonpathogenic

virus named after its host. Here, we image Cafeteria roenbergensis in culture by two-photon excited NADH autofluorescence at video-rate (30 frame/s), and the movement of Cafeteria roenbergensis is recorded in live videos. The result demonstrates the potential use of two-photon microscopy to investigate the interaction between Cafeteria Roenbergensis with virus CroV. The long-term goal is to study specific viral-host interaction process which could lead to important medical applications.

8944-14, Session 3

Real-time in vivo imaging of circulating lymphocytes in high endothelial venules of lymph node

Kibaek Choe, Yoonha Hwang, Howon Seo, Eunjoo Song, Pilhan Kim, KAIST (Korea, Republic of)

Lymph nodes (LNs) distributed over whole body are major checkpoints for circulating T lymphocytes to recognize foreign antigens. High endothelial venules (HEVs) in LN facilitate effective recruitment of circulating T lymphocytes from the blood into the LN. There have been many studies to visualize the lymphocytes trafficking across LN-HEV from blood circulation. However, clear visualization of dynamic behaviors of rapidly flowing lymphocytes in HEV and their transendothelial/perivascular migration have not been achieved due to insufficient spatiotemporal resolution and a lack of appropriate in vivo labeling method of HEV-endothelial cells and perivascular region. In this work, we adapted a custom-design video-rate triple-color laser scanning confocal microscopy system to track rapidly flowing T lymphocytes in HEV in real time in vivo. HEVs in LN were clearly identified in vivo with its distinctive cuboidal morphology of endothelial cells fluorescently labeled by intravenous injection of Alexa488-conjugated anti-CD31 antibody (green). By visualizing the adaptively transferred T lymphocytes and red blood cells (RBCs) labeled with CMTMR (red) and DiD (near infra-red) respectively, we successfully analyzed flowing behaviors of T lymphocytes in comparison with RBCs in HEVs. In addition, for the first time to our knowledge, the paracellular transendothelial migration of T lymphocytes squeezing in between cuboidal-shaped endothelial cells of HEV was clearly visualized in vivo. After the transenothelial migration, the T lymphocytes searched appropriate exit site of perivascular channels surrounded by fibro-reticular cells labeled with Alexa647-conjugated anti-ER-TR7 antibody (near infra-red). Furthermore, we also visualized B lymphocyte trafficking across HEV to compare with T lymphocyte behaviors.

8944-15, Session 4

Phantom Study Based on a High-Energy In-line Phase Contrast Tomosynthesis Prototype

Di Wu, The Univ. of Oklahoma (United States); Aimin Yan, The Univ. of Alabama at Birmingham (United States); Yuhua Li, The Univ. of Oklahoma (United States); Wei R. Chen, Univ. of Central Oklahoma (United States); Xizeng Wu, The Univ. of Alabama at Birmingham (United States); Hong Liu, The Univ. of Oklahoma (United States)

The objective of this research is to demonstrate an in-line phase contrast tomosynthesis prototype operated under high x-ray tube voltage, and a phantom study was conducted to characterize the potentials of this system. The prototype is based on an in-line phase contrast system accompanying with digital tomosynthesis imaging mechanism; and the tube voltage is operated at 120 kVp. A phantom study was conducted by using a custom-designed fish bone phantom to demonstrate the ability of this imaging system in edge enhancement and noise suppression. As the result, edge enhancement could be observed on the in-plane slices by plotting and comparing the intensity profiles with DTS images. As employing phase retrieval method onto the original angular projections could dramatically improve the image quality in edge enhancement, 3D imaging box was preliminarily constructed by using reconstructed in-plane slices acquired with PAD phase retrieval. As expected, high-energy in-line phase contrast tomosynthesis imaging system shows its potentials in edge enhancement and noise suppression by introducing phase retrieval method. Dose studies and perfecting photon energies and phantom designs will be our future interest.

8944-16, Session 4

Background estimation methods for quantitative X-ray fluorescence analysis of gold nanoparticles in biomedical applications

Liqiang Ren, Di Wu, Yuhua Li, The Univ. of Oklahoma (United States); Wei R. Chen, Univ. of Central Oklahoma (United States); Hong Liu, The Univ. of Oklahoma (United States)

Accurate background estimation to isolate the fluorescence signals is an important issue for quantitative X-ray fluorescence (XRF) analysis of gold nanoparticles (GNPs). Though a good estimation can be obtained experimentally through acquiring the background spectrum of water solution, it inevitably leads to unnecessary second exposure in reality. Thus, several numerical methods such as trapezoidal shape estimation, interpolation by polynomial fitting and SNIP (Statistics sensitive Nonlinear Iterative Peak-Clipping) algorithm are proposed to achieve this goal. This paper aims to evaluate the estimation results calculated by these numerical methods through comparing with that acquired using the experimental way, in term of mean squared error (MSE). Four GNP/water solutions with various concentrations from 0.0% to 1.0% by weight are prepared. Then, ten spectra are acquired for each solution for further analysis, under the identical condition of using pencil beam x-ray and single spectrometer. Finally, the experimental and numerical methods are performed on these spectra within the optimally determined energy window and their statistical characteristics are analyzed and compared. These numerical background estimation methods as well as the evaluation methods can be easily extended to analyze the fluorescence signals of other nanoparticle biomarkers such as gadolinium, platinum and Barium in multiple biomedical applications.

8944-17, Session 4

Anti-tumor immunological effects induced by laser-nanotechnology

Wei R. Chen, Univ. of Central Oklahoma (United States); Robert E. Nordquist, Wound Healing of Oklahoma, Inc. (United States); Feifan Zhou, South China Normal Univ. (China); Hong Liu, The Univ. of Oklahoma (United States); Tomas Hode, Immunophotonics, Inc. (United States)

Nanomaterials and lasers are two major scientific innovations in the past several decades. The biomedical applications of these two novel techniques hold great promises for drug delivery, diagnosis and disease treatment. We combined these two for cancer treatment. Specifically, we utilize the special absorption spectrum of carbon nanotubes for selective photothermal interactions to induce targeted tumor cell damages. We also introduced an immunostimulant, glycated chitosan (GC), used in synergism with the laser-nanotechnology treatment. The combined thermal and immunological interactions showed significant effect in treating animal tumors. In our recent studies, the laser-nano treatment of a tumor on one side of a mouse significantly affected the growth of the untreated tumor on the opposite side, a clear sign of induced systemic anti-tumor effects. Furthermore, laser-nano treatment apparently lost its efficacy when we used tumor-bearing nude mice, indicating the necessity

of an intact host immune system required for the laser-nano treatment of tumors.

8944-18, Session 4

Special antitumor immune effects of laser immunotherapy with SWNT-GC

Feifan Zhou, Sheng Song, South China Normal Univ. (China); Wei R. Chen, Univ. of Central Oklahoma (United States)

Anti-tumor immunological response induced by local intervention is ideal for treatment of metastatic tumors. Laser immunotherapy was developed to combine photothermal interaction with immunological stimulation (glycated chitosan, GC) for cancer treatment. Now we construct a complex of GC with SWNT. Laser+SWNT-GC resulted in effective tumor suppression in an animal mammary tumor model. To further study mechanisms of the anti-tumor immunity induced by laser+SWNT-GC, passive adoptive immunity transfer was performed using splenocytes as immune cells. Furthermore, cytotoxicity and TNF? secretion by splenocytes from treated mice also indicated that laser+SWNT-GC induced immunity was tumor-specific. The high level of T cells infiltrating tumors after laser+ SWNT-GC treatment further confirmed the special antitumor immune response. Therefore, laser+ SWNT-GC could prove to be a promising selective local treatment modality that induces systemic anti-tumor response.

8944-19, Session 4

Enhanced photo-transfection efficiency on graphene coated substrates

Patience Mthunzi, Council for Scientific and Industrial Research (South Africa); Kuang He, Univ. of Oxford (United Kingdom); Sandile Ngcobo, Council for Scientific and Industrial Research (South Africa); Jamie Warner, Univ. of Oxford (United Kingdom)

Graphene, an atomic-thick sheet of carbon atoms has been reported by Nayak et al, 2011 [1] as one of the promising biocompatible scaffolds that promotes cellular proliferation in human mesenchymal stem cells. On the other hand, different mammalian cell lines including the induced pluripotent stem cells exhibited an accelerated proliferation rate when cultured on graphene or graphene oxide coated substrates [2]. These findings provide strong motivation to explore the full capability of graphene in further pluripotent stem cell research activities as there exists an urgent requirement to preserve their therapeutic potential. This therefore calls for non-invasive procedures for handling stem cells in-vitro. For example, resent literature has shown successful laser light driven transfection in both multipotent and pluripotent stem cells [3 - 6]. In order to explore the non-invasive nature of optical transfection alongside biocompatible qualities of grapheme, in this work we investigated the impact of optically transfecting mouse embryonic stem (mES) cells plated on graphene coated sample chambers. Using Chinese Hamster Ovary cells (CHO-K1), we further studied the influence of graphene on cell viability as well as cell cytotoxicity through assessing changes in levels of mitochondrial adenosine triphosphate (ATP) activity and the release of cytosolic lactate dehydrogenase (LHD) respectively. Our results showed that compared to those treated on plain glass, CHO-K1 cells optically treated while plated on graphene coated substrates exhibited a higher production of ATP and a milder release of LDH. In addition there was enhanced photo-transfection efficiency in both CHO-K1 and mES cells irradiated on graphene sample chambers.

8944-20, Session PMon

Effect of High-dose laser (532 nm) Irradiation on rat synovial fibroblasts

Leiting Pan, Shuying Yang, Cunbo Li, Xinzheng Zhang, jingjun Xu, Nankai Univ. (China)

Synoviocyte hyperplasia is important for rheumatoid arthritis, therefore, potentially an target for therapeutics. Laser has been shown to some beneficial or bad effects on various cell types. In this study, rat synovial fibroblasts were exposed to green semiconductor laser (532 nm) to investigate the effects of laser irradiation on cell viability as tested by trypan blue exclusion. Synovial fibroblasts were obtained from rats with collagen-induced arthritis. In all experiments, green laser was focused on the samples by a 20? objective. Cells were put into incubator for resting with 1 hour after laser irradiation. Then, trypan blue was used to tested cell viability. Synovial fibroblasts were irradiated by the continuous green laser at 450 J/cm2, 750 J/cm2, 1350 J/cm2 and 2250 J/cm2, respectively. Interestingly, such high-dose laser Irradiation had no effect on the cell viability, whereas other researchers suggested that low-dose light irradiation could resulted in significant physiological effects in cells. Furthermore, high-dose laser Irradiation did not induce cell death in HeLa cells. We believe that these results might be helpful for the clinical application of laser irradiation.

8944-21, Session PMon

Prevention of nano-liposomal resveratrol on sodium nitroprusside-induced rabbit chondrocytes apoptosis

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Osteoarthritis (OA) is a progressive and functionally debilitating disease characterized by apoptosis of chondrocyte. The chondrocyte is the only cell type present in mature cartilage, and solely responsible for the production and maintenance of the extracellular matrix, which is accounted for the mechanism of OA. Resveratrol (RV, trans-3,4',5-trihydroxystilbene) is a natural polyphenol compound that is considered to be anti-oxidative, anti-inflammatory, anti-aging, anti-cancer, anti-viral and recently is reported to has the activity of anti-OA. In this study, articular cartilage was biopsied from the joints of 6 weeks old New Zealand rabbit, and exposure of chondrocytes to sodium nitroprusside(SNP) induced a up-regulation of the production of reactive oxygen species (ROS) and a remarkable reduction of cell viability. We found that although RV prevented chondrocyte from SNP-induced apoptosis, RV is slightly soluble in water. RV is generally dissolved in dimethylsulfoxide(DMSO) which has cytotoxicity to chondrocytes. Then we here used nanoliposomes to load RV to solve these problems. Nanoliposome is an ideal carrier for loading drugs to improve the solubility and bioavailability without cytotoxicity. Cell Counting Kit (CCK-8) assay revealed that nanoliposomal RV remarkably suppressed the SNP-induced cytotoxicity toward chondrocytes. Moreover, we used flow cytometry (FCM) to assess the degree of cell protection by Annexin-V/PI double staining.

8944-22, Session PMon

Application of OCT elastography for diagnosis of thyroid hydatoncus

Zhifang Li, Xiaona Lin, Hui Li, Fujian Normal Univ. (China); Wei R. Chen, Department of Engineering and Physics, University of

Central Oklahoma (United States)

We present an optical technique to image elastic properties of the human thyroid with suspected cysts utilizing optical coherence elastography. Combining the optimized kernel size for 2D normalized cross-correlation with the optimum scale for wavelet differentiation method, four types of images including axial/lateral displacement, axial strain elasogram, modulus and Poisson's ratio elastograms were obtained to characterize elasticity variations of the suspected cysts. Results suggest that the herein presented method enables to distinguish benign lesions qualitatively. Thus, elastic properties imaging based on optical coherence elastography shows great promise for the detailed characterization of lesions and preliminary diagnosis of human thyroid diseases.

8944-23, Session PMon

Influence of optical parameters of tissue on photoacuostic signal: a pilot study

Wenming Xie, Yubin Liu, Zhifang Li, Hui Li, Fujian Normal Univ. (China)

In last decade, Photoacoustic imaging as a promising technology made a great progress for biomedical application. Generation of a Photoacoustic signal is the result of interaction between light and tissue. A photoacoustic signal should be affected by optical parameters of tissue. Many documents alluded to the relation between an absorption coefficient and the photoacoustic signal, but the scatter coefficient seldom be mentioned. In this paper, we proposed a method to decompose the detected photoacoustic signal. We discussed about the photoacoustic signal affected by the optical parameters in a slab sample. Preliminary experimental results show the frequency, phase and amplitude of photoacoustic was determinated by the optical parameters of tissue.

8944-24, Session PMon

Nanotechnology and phototherapy for cancer treatment

Joseph T. Acquaviva III, Univ. of Central Oklahoma (United States); Feifan Zhou, South China Normal Univ. (China) and Institute of Laser Life Science (China); Wei R. Chen, Univ. of Central Oklahoma (United States); Tomas Hode, Immunophotonics, Inc. (United States); Hong Liu, The Univ. of Oklahoma (United States)

For the treatment of metastatic cancers, a novel therapy has been developed: laser immunotherapy. Clinical trials have confirmed laser immunotherapy as an effective treatment for patients with late-stage metastatic breast cancer and melanoma. The combination of phototherapy (laser irradiation) and immunological stimulation produces a synergistic effect capable of inducing a systemic anti-tumor response. By synthesizing a new solution, immunologically modified carbon nanotubes, laser immunotherapy has been enhanced. Single-walled carbon nanotubes (SWNTs) were combined with the immunostimulant glycated chitosan (GC). This novel solution, SWNT-GC, has been developed as an enhanced light absorbing agent for selective photothermal interaction and as a vehicle for GC to enter cells. An increase in the concentration of SWNT-GC proved capable of inducing lower cellular viability. Additionally, tumors treated with phototherapy and SWNT-GC activated immune cells against cancer. Our results indicated that immunologically modified carbon nanotubes, used in conjunction with phototherapy, could be a systemic method for treating metastatic cancers.

8944-25, Session PMon

In vivo, label-free, and noninvasive detection of melanoma metastasis by photoacoustic flow cytometry

Rongrong Liu, Shanghai Jiao Tong Univ. (China); Cheng Wang, Univ. of Shanghai for Science and Technology (China); Cheng Hu, Shanghai Jiao Tong Univ. (China); Xueding Wang, Univ. of Michigan (United States); Xunbin Wei, Shanghai Jiao Tong Univ. (China)

Melanoma is the most serious type of skin cancer in the world, and accounts for about 80% of deaths of all skin cancer. Here, we have used an emerging technique, namely in vivo photoacoustic flow cytometry (PAFC) to evaluate the therapeutic effects of the treatment on metastatic melanoma. The in vivo flow cytometry (IVFC) has shown a great potential for detecting circulating tumor cells quantitatively in the bloodstream. Compared with fluorescence-based in vivo flow cytometry (IVFC), this PAFC technique can be used for in vivo noninvasive and label-free detection of circulating tumor cells (CTCs) in circulation.

8944-26, Session PMon

Simultaneous delivery of melittin and paclitaxol for synergistic cancer chemoimmunotherapy based on peptide-controlled lipid nanoparticles

Chuan Huang, Honglin Jin, Yuan Qian, Zhihong Zhang, Britton Chance Ctr. for Biomedical Photonics (China)

Cancer chemotherapy drugs have been considered immune suppressant for the side effects on immune cells for a long time. However, more recent studies have shown that some of the chemotherapeutic drugs can also promote immune activation. Therefore, it is generally accepted that cancer chemoimmunotherapy, the combination of chemotherapy and immunotherapy, is gradually becoming a new type of cancer treatment modality. Melittin as a cytolytic peptide has shown some promise as a cancer chemoimmunotherapeutic agent, but in vivo applications of melittin are limited due to its main side effect, hemolysis, which is especially pronounced following intravenous administration. Here we show a multi-functional lipid nanoparticle, which is able to stably load the melittin peptides onto its lipid monolayer and incorporate the optical probes and another chemoimmunotherapy drug paclitaxol in the core. This nanoparticle effectively reduced the hemolytic properties of melittin used in vivo. The simultaneous loading of melittin and paclitaxol resulted in a synergistic therapeutic effect to tumor cells due to the induction of cell lysis by melittin on the membrane and apoptosis by paclitaxol in the cytoplasm. Moreover, melittin and paclitaxol activated the immune systems in tumor microenvironment through the release of tumor antigen and activation of immune cells, and these processes further potentiated the efficacy of cancer therapy. Additionally, the real-time fluorescence imaging results confirmed the antitumor agents efficiently induced melanoma cell death, and the behaviors of immune cells were more active. Thus, excellent properties of simultaneous delivery nanoparticles give it potential clinical applications in the cancer chemoimmunotherapy through intravenous administration.

8944-27, Session PMon

In vivo visualizing effector function of mixed cytokines preactivated NK in combined chemoimmunotherapy for B16 melanoma

Fei Yang, Lili Zhou, Shun Liu, Qingming Luo, Zhihong Zhang,

Britton Chance Ctr. for Biomedical Photonics (China)

NK cell-based immunotherapy is promising to suppress tumor growth in combination with chemical drugs. However, the curative efficiency by this approach is generally hampered by the transient effector function of NK cells. NK cells preactivated by mixed cytokines have been shown to be “memory-like”, a characteristic of adaptive immunity. This “memory-like” property endowed NK cells sustained effector function in vivo. However, it is unknown how exactly these preactivated NK cells behave in tumor microenvironment following adoptive transfer. Our study investigated the long-term dynamics of mixed cytokines-preactivated NK cells in B16 melanoma micro-environment and compared NK dynamics under conditions with/without combined chemoimmunotherapy by the means of intravital microscopy. In our study, we obtained NK cells of nearly 20 ?m in diameter with potent cytotoxicity, which could kill more than 50% of the YAC-1 cells even at the effector/target ratio of 1:3. In response to immune-regulatory drugs, B16 melanoma cells became more sensitive to preactivated NK cells. In combination with Treg and MDSC depletion by cyclophosphamide and 5-fluorouracil, adoptively transferred NK cells infiltrated inside established solid tumors more effectively than controls and showed sustained effector function at 7 days after transfer. Under this therapeutic condition, preactivated NK cells showed enhanced motility and attacked tumor cells more frequently. Altogether, our results showed how these chemical drugs take effect on the dynamics of adoptively transferred NK cells and established an approach for direct killing of tumor cells by preactivated NK cells in vivo.

8944-28, Session PMon

Metabolic imaging of tumors treated by KillerRed fluorescent protein-based photodynamic therapy

Shuang Sha, Lingsong Qin, Anle Wang, Honglin Jin, Zhihong Zhang, Britton Chance Ctr. for Biomedical Photonics (China)

KillerRed is a unique red fluorescent protein exhibiting excellent phototoxic properties. It has the ability to produce reactive oxygen species (ROS), for killing tumor cells in vitro upon laser irradiation and has the potential to act as a photosensitizer in the application of tumor therapy. Here, we investigated the effects of KillerRed-based photodynamic therapy (PDT) on tumor growth in vivo and examined the subsequent tumor metabolic states including the changes of pyridine nucleotide (PN) and flavoprotein (Fp), two important metabolic coenzymes of tumor cells. A lentivirous vector lentihiko was used to transfect mitochondria-located KillerRed to HT1080 cells. The cell line stably expressing KillerRed was selected and implanted into nude mice to establish subcutaneous tumor models. Results show that the tumors scabbed in response to 150mW/cm2 561nm laser irradiation for 30 min. However, similar phenomenon was not observed in the control groups. After daily treatment for a week, the tumors were subsided. A home-made cryo-imaging redox scanner was used to measure intrinsic fluorescence and exogenous KillerRed fluorescence signals in tumors. The results show that the flavoprotein was remarkable elevated but the PN was decreased with concomitant photobleaching of KillerRed fluorescence after irradiation, suggesting that flavoprotein and PN were oxidized in the courses of KilleRred-based PDT. Together, this study demonstrates the effectiveness of KillerRed as an alternative PDT agent and provides useful information regarding the tumor metabolic states following KilleRred-based PDT

8944-29, Session PMon

Mechanistic studies of systemic immune responses induced by laser-nanotechnology

Wei R. Chen, Univ. of Central Oklahoma (United States); Robert E. Nordquist, Wound Healing of Oklahoma, Inc. (United States); Tomas Hode, Immunophotonics, Inc. (United States); Hong Liu, The Univ. of Oklahoma (United States); Feifan Zhou, South China Normal Univ. (China)

With the help of the specific absorption spectrum of carbon nanotubes, we achieved selective photothermal tumor cell destruction, particularly using a near-infrared laser to reduce potential damage to untargeted tissues. Combined with immunological stimulation, using a novel adjuvant, we also observed the anti-tumor immune responses when treating animal tumors using the laser-nano treatment. In fact, the local application of laser-nano-immunotherapy appeared to result in a systemic curative effect. In our mechanistic study, we found that the laser-nano-immuno treatment can activate antigen-presenting cells, such as dendritic cells (DCs). More importantly, the uptake and presentation of antigens by these antigen presenting cells were significantly enhanced, as shown by the strong binding of tumor cells and DCs as well as the proliferation of T cells caused by the DCs after the DCs had been incubated with laser-nano-immuno treated tumors. These cellular observations provide evidence that a systemic anti-tumor immune response was induced by the combination of laser and nanotechnology.

8944-30, Session PMon

Interphase Fluorescence in situ Hybridization Signal Detection by Computing Intensity Variance Along the Optical Axis

Zheng Li, The Univ. of Oklahoma (United States); Bin Zheng, university of oklahoma (United States); Liqiang Ren, Hong Liu, The Univ. of Oklahoma (United States)

Fluorescence in situ Hybridization (FISH) technology is a commonly used tool to detect chromosome aberrations, which are often pathological significant. Since manual FISH analysis is a tedious and time-consuming procedure, reliable and robust automated image acquisition and scanning microscopic systems are in demand for efficiency improvement of FISH analysis. Under high magnification objective lenses such as 60x and 100x, the depth of field will often be too small and the FISH probes may not always lie in the same focal plane. A statistical variance based FISH probe detection scheme is developed in order to address this problem. A stack of image slices are acquired, in steps of size d, alone the z-axis. Statistical variance is calculated alone the z-axis to form a 2-D matrix. Since pixels shift dramatically to high intensity at FISH probe location, the probes will manifest high peak values in the matrix. A computer-aided detection scheme based on top-hat transform is applied to the matrix to detect FISH probe signals. This study demonstrates a simple and robust method for FISH probe detection as well as a way of 2-D representation of 3-D data.

8944-31, Session PMon

A mathematical model of the dynamics of anti-tumor laser immunotherapy

Sean M. Laverty, Bryan Dawkins, Univ. of Central Oklahoma (United States)

We study a general model of anti-cancer laser immunotherapy, consisting of a system of several differential equations that describe population dynamics of host cells and other immune system components. Using the model, we examine the roles of immunoadjuvant and laser treatment in generating anti-cancer cellular immunity, and we mathematically characterize conditions under which treatment is successful (e.g., cancer is controlled or eliminated). In this analysis, we take a general approach to studying cancer-immune dynamics. We explore the theoretical success of laser immunotherapy when applied to a variety of metastatic cancers, by choosing underlying model of cancer growth rate from commonly

published functional forms in the cancer and cancer immunotherapy literature. We seek to produce a theoretical framework that identifies what growth characteristics make a given cancer more conducive to control by laser immunotherapy.

8944-32, Session PMon

Photostimulation regulates actin filament rearrangements by activating PI3K signaling pathway in macrophage

Zhijin Fan, Cuixia Lu, Sheng Song, Feifan Zhou, South China Normal Univ. (China)

Macrophage phagocytosis is critical for defense against pathogens, among which many steps are dependent on cytoskeleton. Low power laser irradiation (LPLI) has been found to produce photobiological effects with evidence of interference with immunological functions. However, the machanism is largely unknow. In this study, we focused our attention on the effects of He-Ne laser on the actin filament rearrangements of macrophages by using confocal laser scanning microscopes (LSM). After irradiation at the dose of 2 J/cm2 with He-Ne laser (632.8 nm, 10 mw), the cells were treated with Phalloidin staining and then subjected to confocal observation. The results showed that LPLI led to an increase in the F-actin content of the murine macrophage-like cell line RAW264.7. In addition, we demonstrated that the actin polymerization induced by LPLI was PI3K-dependent. Taken together, our results indicated that LPLI enhanced the F-actin content of macrophage through PI3K-dependent pathway, which provided a theoretical base for the clinical use of the He-Ne laser.

8944-33, Session PMon

Low-power laser irradiation (LPLI) attenuates microglial cytotoxicity through the activation of Src pathway

Sheng Song, South China Normal Univ. (China); Wei R. Chen, Univ. of Central Oklahoma (United States); Feifan Zhou, South China Normal Univ. (China)

It has been known for a long time that microglial activation plays an important role in the pathology of neurodegenerative diseases. Once activated, they have macrophage-like capabilities, which can be detrimental by producing proinflammatory and neurotoxic factors including cytokines, reactive oxygen species (ROS) and nitric oxide that directly or indirectly cause neurodegeneration. Therefore, the regulation of microglial-induced neuroinflammation is considered a useful strategy in searching for neuroprotective treatments. In this study, our results showed that low power laser irradiation (LPLI) (20 J/cm2) could suppress microglial-induced neuroinflammation in LPS-activated microglia. We found that LPLI-mediated neuroprotection was achieved by activating tyrosine kinases Src, which led to MyD88 tyrosine phosphorylation, thus impairing MyD88-dependent proinflammatory signaling cascade. Our research may provide a feasible therapeutic approach to control the progression of neurodegenerative diseases.

8944-34, Session PMon

Combination therapy of EGFR gene and photodynamic therapy to enhance oral cancer treatment efficacy

Chia-Hsien Yeh, Chung Yuan Christian Univ. (Taiwan); Leaf Huang, UNC Eshelman School of Pharmacy (United States); Yih-Chih Hsu, Chung Yuan Christian Univ. (Taiwan)

Photodynamic therapy (PDT) is a kind of treatment for cancer. The principle is photosensitizer absorbed by cancer cells and then cell irradiated with light, the photosensitiser is converted from the ground state to active state. It causes tumor cell death by generated free radicals and singlet oxygen. Overexpression of human epidermal growth factor receptor (EGFR) has been detected in oral cancer cells. Combination treatment of photodynamic therapy and EGFR-targeting agents are potential therapeutic modalities for treating oral cancer based on in vitro study.

Conference 8945: Design and Performance Validation of Phantoms Used in Conjunction with Optical Measurement of Tissue VI

BiOS SPIE Photonics West

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8945 Design and Performance Validation of Phantoms Used in Conjunction with Optical Measurement of Tissue VI

8945-1, Session 1

Use of a standard reference material for validating near infrared optical imaging tissue phantom

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Fluorescence imaging in the near infrared is an increasingly valuable tool in clinical diagnostics. As with other fluorometric instruments, characteristics such as spectral responsivity, linearity, spectral irradiance at the sample and lower limit of analyte detectability as well as daily performance are needed measurements. For this purpose, tissue phantoms are developed to calibrate the imaging instrument in order to facilitate refinement of the imaging system and operations protocol. These tissue phantoms which are used as working standards are often designed to mimic specific tissue optical properties. These working standards in turn need to be validated. Doped glasses with luminescence in the near infrared are available as relative intensity standards for Raman spectroscopy. Here we demonstrate the use of these existing standard reference luminescent optical glass for validating tissue phantoms.

8945-2, Session 1

Polymer microfluidic phantoms for characterization of fluorescence laminar optical tomography (FLOT) system

Jianting Wang, Chao-Wei Chen, Anthony Fouad, Siddarth Plakkot, Hyounguk Jang, Yu Chen, Univ. of Maryland, College Park (United States)

Fluorescence laminar optical tomography (FLOT) is a new medical imaging modality that provides high-resolution, depth-resolved information. It uses a focused light beam to scan over the tissue surface, and the fluorescence is detected at different distance from the scanning beam. Monte Carlo simulation is used to model the photon migration in the turbid tissue to resolve the distribution of fluorophores. The data acquisition and reconstruction process are complex and varied from system to system, therefore thorough characterization and validation with phantom studies are highly desired. Here we designed and fabricated PDMS phantoms with sub-millimeter channels using photolithography and molding of PDMS. The turbidity of the phantoms was tuned by adding BaSO4 powder. The depth of the channels was varied. The geometry of the channels was characterized using light microscopy, optical coherence tomography (OCT) and microCT.

Fluorescent dye solutions were injected in the channels and the phantoms were imaged by our angled-FLOT (aFLOT) system. The performance of the system to accurately resolve the geometry of the channels and to quantify the fluorescent dye in the channels was characterized and optimized. The aFLOT system was then applied for imaging of mouse colon tissue with colorectal cancer (CRC). A Cathepsin B-triggered fluorescent probe (Ex/Em 680/700 nm) was used as the contrast agent. The imaging results were verified by sectioning and histological analysis of the tissues. The aFLOT system demonstrated its ability to image the CRC in 3D with 100-200 μm resolution over ~2 mm depth, showing the potential of providing real-time assessment of the morphology of the tumor.

8945-3, Session 1

Estimating the spatial resolution of fNIRS sensors for BCI purposes

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Differential near infrared sensors recently sparked a growing interest as promising measuring modality for brain computer interfacing. In our study, we present the design and characterization of novel, differential functional NIRS sensors, intended to record hemodynamic changes of the hand-area of the human motor cortex during motor imagery tasks.

We report on the spatial characterization of a portable multi-channel NIRS system with one module consisting of two central LEDs (770nm and 850nm) and four symmetric pairs of radially aligned photodiodes (PD) resembling a plus symbol. The other sensor module features four similar, differential light paths crossing in the center of a star.

Characterization was performed on a concentric, double beaker functional phantom, featuring a PBS/intralipid/blood mixture (97%/1%/2%). In extension of previous work, the inner, oxygenated beaker was covered by neopren sleeves with holes of decreasing sizes, thus giving an estimate on the spatial limits of the NIRS sensor‘s measurement volume.

The star shaped sensor module formed a diffuse focus of around 1.5 cm in diameter at 1.4 cm depth, whereas the plus shaped arrangement hinted a concentric ring of four separate regions of interest.

The systems measurement sensitivity could be improved by removing ambient light from the PD by adequate filtering.

Altogether, we conclude, that both our novel fNIRS design as well as its electronics perform well in the double layered oxygenation phantom and are thus ready be tested in vivo.

8945-4, Session 1

Development and characterization of a brain tumor mimicking fluorescence phantom

Neda Haj-Hosseini, Linköping Univ. (Sweden); Benjamin Kistler, Univ. of Northwestern Switzerland (Switzerland) and Linköping Univ. (Sweden); Karin Wårdell, Linköping Univ. (Sweden)

Fluorescence guidance using 5-aminolevulinic acid (5-ALA) for brain tumor resection is a recent technique applied to the highly malignant brain tumors. Five-ALA accumulates as protoporphyrin IX fluorophore in the tumor cells in different concentrations depending on the tumor environment and cell properties. Our group has developed a fluorescence spectroscopy system which has shown improvement of fluorescence detection and quantification that preliminarily correlates with tumor malignancy grade during surgery. However, quantification of fluorescence is affected by several factors including the initial fluorophore concentration, photobleaching due to operating lamps prior to the measurements and attenuation from the tissue. Accordingly, an optical phantom was developed to enable controlled fluorescence measurements and evaluation of the system outside of the surgical procedure. The phantom mimicked the optical properties of the highly malignant brain tumor at the specific fluorescence excitation wavelength

when different concentrations of the fluorophore were included in the phantom. To allow evaluation of photobleaching, diffusion of fluorophore molecules in the phantom was restricted by solidifying the phantoms. Morover, a model for tissue autofluorescence was added. The fluorescence intensity's correlation with the fluorophore concentration in addition to the photobleaching properties were investigated in the phantoms and were compared to the clinical data measured on brain tumor.

8945-5, Session 1

The development of a simplified epithelial tissue phantom for the evaluation of an autofluorescence mitigation algorithm

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The incidence of Barrett's esophagus (BE) is on the rise; as the non-cancerous precursor state to esophageal adenocarcinoma (EAC), accurate BE staging is critical in improving disease outcome. Diagnosis can be enhanced through assessment of overexpressed biomarkers specific to disease state. Such biomarkers include Insulin-like growth factor II mRNA-binding protein 3 (IMP3), Cyclophilin A/Peptidylprolyl isomerase A (CYPA/PPIA), and Alpha-methylacyl-CoA racemase (AMACR). Peptides conjugated to fluorescent dyes can be used to target these biomarkers. An ultrathin, flexible, multimodal scanning fiber endoscope (SFE) can both excite and detect fluorescently labeled targets. Autofluorescence (AF) is a confounder in fluorescence imaging when using the FDA approved FITC fluorophore and an AF mitigation algorithm is needed to enhance low target to background (TB) ratios. To verify the algorithm, a simplified epithelial tissue phantom containing collagen, an abundant endogenous, submucosal fluorophore was seeded with BE cell lines, CP-A, CP-B, CP-C, and CP-D. IMP3, CYPA/PPIA, and AMACR were immunofluorescently labeled with FITC. AF mitigation was applied in real time to improve FITC TB ratios.

8945-6, Session 2

CI Slide: calibration slide for quantitative microscopy imaging in absorbance

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New imaging technologies are fundamentally changing the field of pathology As any new medical technologies, digital pathology faces numerous challenges; e.g. multiple system brands can be installed in the same lab, studies can be based in different multicenter and can last for years. Standardization, Calibration protocols, quality control and quantitative assessment of these different systems have not yet been really investigated. We have design a new Calibration Imaging Slide (CI Slide) specifically to measure the characteristics of old or new imaging systems or scanners. The layout of the slide consists of 132 boxes with the length of 1.6 mm, containing objects of known morphologic and photometric characteristics. One hundred and twelve boxes include different permutations of circles, ovals, and square. The circles have different radii, radius/pitch ratio and step transmission. The ovals have different sizes and orientation. The squares are consistent in the size and orientation but have different step transmissions values. Ten boxes contain two resolution test targets: USAF target and Siemens star. The last 10 boxes are blank boxes with different transmission values. Three CI slides were scanned and imaged on two different commercial whole-slide scanners and two high resolution imaging systems. All the objects on scanned images were segmented and about 200 features (morphologic and architectural) were measured with our in-house image processing software. We will present the results of the multilevel statistical comparisons between the different systems. A specific protocol for system calibration using the CI slide will also be discussed.

8945-7, Session 2

Construction of a digital and physical mouse model aimed at the study of electrical shock

Thu Ahn Nguyen, The Catholic Univ. of America (United States); Jessica C. Ramella-Roman, The Catholic Univ. of America (United States); Jeffrey W. Shupp, MedStar Washington Hospital Ctr. (United States); Lauren Moffatt, MedStar Washington Hospital Ctr. (United States); Jessica C. Ramella-Roman, The Catholic Univ. of America (United States)

Optical methods have been used to investigate electrical injury on animal models such as live mice, rats, and rabbits. Here we introduce a completely digital phantom of a mouse, and its physical 3D reconstruction, with the aim of investigating electrical injury through spectroscopic imaging techniques.

The basis of our phantom is a three-dimensional digital mouse reconstructed from co-registered computed tomographic images and cryosections by a different group. Image processing algorithms were applied to make the model suitable to Finite Element Analysis of thermal and electrical transport. Our digital model is capable of simulating temperature, voltage, current changes along the animal body during and after electrical shocks.

The physical realization of the model is achieved with 3D printed molds of the mouse skin, and 4 internal organs. The molds can be used to create gels of these organs with different optical properties. The combination of digital and physical phantoms can be used to study of high voltage DC shock and its impact on superficial tissue.

8945-8, Session 2

3D printing method for freeform fabrication of optical phantoms simulating heterogeneous biological tissue

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The performance of biomedical optical imaging devices heavily relies on appropriate calibration. However, many of existing calibration phantoms for biomedical optical devices are based on homogenous materials without considering the multi-layer heterogenous structures observed in biological tissue. Using such a phantom for optical calibration may result in measurement bias. To overcome this problem, we propose a 3D printing method for freeform fabrication of tissue simulating phantoms with multi-layer heterogenous structure. The phantom simulates not only the morphologic characteristics of biological tissue but also absorption and scattering properties. The printing system is based on a 3D motion platform with coordinated control of the DC motors. A special jet nozzle is designed to mix base, scattering, and absorption materials at different ratios. 3D tissue structures are fabricated through layer-by-layer printing with selective deposition of phantom materials of different ingredients. Different base, scattering and absorption materials have been tested in order to optimize the printing outcome. A tissue spectrophotometer is used to test the optical properties of the printed phantoms and compare with those of biological tissue. The goal of this project is to fabricate skin tissue simulating phantoms as a traceable standard for the calibration of biomedical optical spectral devices.

8945-9, Session 3

New polymer-based phantom for photoacoustic imaging

Hideaki Iwazaki, Taiichiro Ida, Yasushi Kawaguchi, Advantest Corp. (Japan); Taiji Nishi, Materialdesign Corp. (Japan); Yukari Tanikawa, Naotaka Nitta, National Institute of Advanced Industrial Science and Technology (Japan)

We will report newly developed polymer-based phantom for photoacoustic imaging systems. Phantoms are important for performance evaluation and calibration of new modalities; however, there is no established method for making phantoms with no long-term change. We have developed skin mimicking phantoms simulating both optical and acoustic properties (i.e. optical scattering and absorption coefficients, and sound velocity). Furthermore, the phantoms are able to give accurate simulation of blood vessels by Inkjet-printing. Newly developed phantoms are consisted of castor oil included acrylic block copolymer and we can fabricate 0.8mm or less thick sheets and pile them using their self- adhesiveness.

8945-10, Session 3

Tissue phantoms for multimodal approaches: Raman spectroscopy and optoacoustics

Merve Wollweber, Christian Suhr, Ann-Kathrin Kniggendorf, Bernhard Roth, Leibniz Univ. Hannover (Germany)

Tissue phantoms with defined reliable and reproducible characteristics are important for the evaluation of biomedical spectroscopic and imaging systems. For multimodal approaches, various demands on tissue phantoms have to be met in order to satisfy the needs of all modalities. We present and discuss a hydrogel phantom that was created for combined Raman spectroscopic and optoacoustic measurements imposing optical as well as acoustic requirements on the phantom. We wanted to create a phantom with defined concentrations of carotenes in an optically scattering and absorbing matrix to test and validate the capability of this combination of methods to quantify specific biomolecules in tissue. The main requirements on this phantom were tissue-like acoustic properties, scalable optical attenuation and incorporation of Raman active target molecules. We used a poly(vinyl alcohol) (PVA) hydrogel as the basic phantom material because of its suitable acoustic and optical characteristics but found that the incorporation of non-polar dyes like carotenoids in the hydrogel is difficult and the optical properties of the biomolecule is significantly altered by the hydrogel molecular environment and production procedure. We show and discuss challenges and solutions in the design process of a suitable tissue phantom.

8945-11, Session 3

On mimicking diffuse reflectance spectra in the visible and near-infrared ranges for tissue-like phantom design

Nicola Debernardi, Paraskevas Dunias, Benno van El, Andrew E. Statham, TNO (Netherlands)

We present a novel methodology to mimic diffuse reflectance spectra of arbitrary biological tissues in the visible and near-infrared ranges. The prerequisite for this method is that the spectral information of basic components is sufficient to mimic an arbitrary tissue.

Using a sterile disposable fiber optic probe the diffuse reflectance spectrum of a tissue (either in vivo or ex vivo) is measured, which forms the target spectrum. With the same type of fiber probe, a wide variety of basic components (ingredients) have been previously measured and all together form a spectral database. A “recipe” for the optimal mixture of ingredients can then be derived using an algorithm that fits the absorption and scattering behavior of the target spectrum using the spectra of the basic components in the database. The spectral mimicking accuracy refines by adding more ingredients to the database.

We demonstrate the validity of the principle by mimicking an arbitrary mixture of components. The method can be applied with different kinds of materials, e.g. gelatins, waxes and silicones, thus providing the possibility of mimicking the mechanical properties of target tissues as well.

The algorithm can be extended from single point contact spectral measurement to contactless multi- and hyperspectral camera acquisition. It can be applied to produce durable tissue-like phantoms for calibration, demonstration or comparison of instruments. The resulting phantoms are portable, durable and provide consistent results over time. They are also more readily available than living tissue or a cadaver; hence they are highly useful when developing new devices.

8945-12, Session 3

Photon path depth in tissue phantoms: a comparison of visible and near-infrared (NIR) wavelengths

Karin M. Asplund, Kenneth A. Schenkman M.D., Wayne A. Ciesielski, Univ. of Washington (United States); Lorilee S. L. Arakaki, Univ. of Washington Medical Ctr. (United States)

Optical spectroscopy is being used increasingly in medical applications to noninvasively investigate tissues below the skin. In order to assure adequate sampling of tissues underlying the skin, photon penetration depth must be known. Photon penetration in tissues has been studied with near-infrared (NIR) light, but experimental study of visible light propagation in tissue has been limited. In this study, a micro-motion system coupled with a reflectance spectroscopy system was used to determine the penetration depth of visible-range and NIR photons (535-800 nm) in phantoms composed of Intralipid and hemoglobin. An absorbing target was placed at intervals of 0.1mm along a 15mm line perpendicular to and bisecting the line between the ends of the source and detector optical fiber bundles. Comparisons between detected light intensities at different target positions were used to determine the most probable photon path depth at 576 nm and at 760 nm. Scattering coefficients, hemoglobin concentrations, and source-detector separations were varied to evaluate their effects on the penetration depth of photons. Results from phantoms containing Intralipid only showed that the most-probable penetration depths at 576 nm were comparable to those at 760 nm. Larger source-detector separations resulted in deeper photon penetration depths for both spectral regions. Changes in scattering over a 4-fold range did not affect the photon path depth appreciably. In the presence of hemoglobin with a source-detector separation of 13 mm, the most probable depth of photon penetration in the visible range was greater than 2.5 mm, and was within 1 mm of the most probable depth of photon penetration in the NIR. This study demonstrates the feasibility of using the visible and NIR regions in transcutaneous reflectance spectroscopy.

8945-13, Session 4

Multilayered disease-mimicking bladder phantom with realistic surface topology for optical coherence tomography

Kristen L. Lurie, Gennifer T. Smith, Saara A. Khan, Audrey K. Ellerbee, Stanford Univ. (United States)

Optical coherence tomography (OCT) has shown potential as a

complementary modality to white light cystoscopy (WLC), the gold standard for imaging bladder cancer. OCT can visualize sub-surface details of the bladder wall, which enables it to stage cancers and detect tumors that are otherwise invisible to WLC. Currently, OCT systems have too slow a speed and too small a field of view for comprehensive bladder imaging, which limits its clinical utility. Validation and feasibility testing of technological refinements aimed to provide faster imaging and wider fields of view necessitates a realistic bladder phantom. We present a novel process to fabricate the first such phantom that mimics both the optical and morphological properties of layers of the normal and diseased bladder wall as they characteristically appear with OCT. The silicone-based phantom comprises three layers: the urothelium, lamina propria and muscularis, each containing an appropriate concentration of titanium dioxide to mimic its distinct scattering properties. As well, the layers each possess a unique surface appearance imposed by a textured mold. Inclusions mimicking the appearance of bladder disease in OCT (e.g., tumors are characterized by the loss of bladder wall layers) are created by excising cured regions of arbitrary shape prior to the formation of anterior layers. This phantom can help to evaluate the efficacy of new OCT systems and software for tumor localization. Moreover, the procedure we have developed is highly generalizable for the creation of OCT-relevant, multi-layer phantoms for tissues that incorporate diseased states characterized by the loss of structure.

8945-14, Session 4

Development of a Widefield Phantom Eye for Retinal Optical Coherence Tomography

Anthony T. Corcoran, Optos plc (United Kingdom) and Univ. of Glasgow (United Kingdom); Gonzalo Muyo, Jano I. van Hemert, Optos plc (United Kingdom); Andrew R. Harvey, Univ. of Glasgow (United Kingdom)

Optical coherence tomography (OCT) has become a standard technology in many clinical examinations of the retina. OCT is used to measure the thickness of retinal features relative to both healthy ranges and prior examinations. Here we introduce a design for a phantom eye that can the measure the performance of ophthalmic OCT scanners in terms of the accuracy of those measurements.

To enable the use of the phantom eye on the latest scanners that can scan to the periphery of the retina, we base our design on the Navarro widefield schematic eye. This facilitates verification procedures that test both the maximum field of view (FOV) of the device and the amount of degradation in the device performance; contrast, axial and transverse resolution that result from a variation in the FOV.

We describe the deviation of the widefield phantom eye to the Navarro schematic eye in terms of (RMS) spot size, optical path length and chief-ray intersection. We have prototyped the phantom eye and verified the structural dimensions of the retinal targets manufactured with multi-material 3D-printing with a high power microscope and a calibrated OCT image. We provide images taken of the phantom eye using the Optos Model E scanner and show it is accuracy and precision for measuring layers of the phantom retina.

We have introduced and prototyped a phantom eye that enables verification of the measurement accuracy of widefield ophthalmic OCT scanners. We also believe this phantom will contribute to maintaining the performance of devices as their FOV increases.

8945-15, Session 4

Phantoms towards dimensional metrology standards for depth-resolving optical medical imagers

Jennifer Field, Robert C. Chang, Stevens Institute of Technology (United States); Daniel Stark, Jeeseong Hwang, Maritoni Litorja, National Institute of Standards and Technology (United States)

An optical tissue phantom fabrication methodology is reported to implement optical coherence tomography (OCT) and near-IR fluorescence imaging as a benchmark towards depth metrology standards of tissue structures to harness the clinical potential of quantitative depth-resolved imaging systems. The fabrication implements a combinatorial approach of layer-by-layer polyelectrolyte multilayer deposition of embedded particle monolayers (scattering microspheres or quantum dots) with replica molding to achieve a monolithic wedge or step-configured axial test target covering a wide spatial frequency to validate the axial resolution and optical contrast in scattering or fluorescence imaging. The results on layer dimensions and axial positions of the embedded particles are independently validated using higher resolution confocal and interferometric microscopy. Ongoing efforts include the use of these calibrated phantoms to evaluate the depth-resolving performance of a variety of optical sectioning imaging platforms including OCT with sub-micron axial resolution and spectrally resolved near-IR fluorescence imaging.

8945-16, Session 4

Diamond-turned calibration standards for optical coherence tomography systems

Amber M. Beckley, Ecole Polytechnique de Montréal (Canada); Mathias Strupler, Sainte-Justine Hospital Research Ctr. (Canada); Jean-Pierre Bouchard, Sylvain Dubois, Ozzy Mermut, INO (Canada); Caroline Boudoux, Ecole Polytechnique de Montréal (Canada) and Sainte-Justine Hospital Research Ctr. (Canada)

As the biomedical community relies more on optical coherence tomography (OCT) for reliable tissue measurement, qualitative and quantitative measures of OCT system optical performance have become increasingly necessary.

We explore designs of diamond-turned calibration targets created to test the optical quality of OCT systems. In-house single point diamond turning and diamond milling allows for custom design of depth and resolution targets. With the ability to create large optical free-form surfaces in a reasonable time frame (up to 50 mm diameter in a few minutes) with precise depth variations on a micron scale with under 20-nanometer roughness, diamond turning is an ideal method to create high quality, durable test plates. Capable of machining in metal or plastic, negative calibration targets can also be made for optical quality molds for tissue phantoms.

With such a calibration plate, we can measure the system axial and lateral resolution, the signal to noise and z-error as a function of depth, distortion, field curvature and the field of view. We present studies of the first such calibration target, which is composed of mirrors at successive 100 micron depths to test OCT system performance. To investigate the imaging quality through a scattering medium, the target was filled with an optical tissue phantom material of absorption coefficient of 15 cm^-1 and reduced scattering coefficient of 0.3 cm^-1 (produced by INO). Tests were conducted for custom and commercial OCT systems. Further designs for resolution tests are also presented.

8945-17, Session 5

Microfluidic channel devices as volumetric measurement phantoms in optical coherence tomography and confocal microscopy

Jeeseong Hwang, Daniel Stark, Darwin Reyes, Michael Halter, National Institute of Standards and Technology (United States)

In optical imaging of a complex vascular network in a human tissue, absorption and scattering light through tissue medium poses a challenge

in quantitative measurements of blood flow characteristics using depth resolving imaging technologies such as optical coherence tomography and confocal microscopy. Microfluidic devices have recently been employed as phantoms simulating a vascular network in a tissue in an effort to enable quantitative imaging. The channel structure and the substances of the liquid flowing through the channel can be designed to mimic capillary blood vessels, and the optical properties of the matrix in which the channels are formed can be matched to those of tissues. However, dimensional calibration in these microfluidic phantoms is challenging as the interrogating light are perturbed by index mismatching interfaces between the matrix and channels filled with a fluid medium with a lower refractive index. Here, we discuss promises and challenges in using microfluidic devices towards dimensional and chemometric metrology standards for depth resolving imagers including optical coherence tomography and confocal microscopy.

8945-18, Session 5

Characterization of a novel time-domain non-contact tissue scanning system

Heidrun Wabnitz, Mikhail Mazurenka, Physikalisch-Technische Bundesanstalt (Germany); Laura Di Sieno, Alberto Dalla Mora, Davide Contini, Gianluca Boso, Alberto Tosi, Politecnico di Milano (Italy); Fabrizio Martelli, Univ. degli Studi di Firenze (Italy); Yoko Hoshi, Tokyo Metropolitan Institute of Medical Science (Japan); Yukari Tanikawa, National Institute of Advanced Industrial Science and Technology (Japan); Rainer Macdonald, Physikalisch-Technische Bundesanstalt (Germany); Antonio Pifferi, Politecnico di Milano (Italy) and CNR, Istituto di Fotonica e Nanotecnologie (Italy)

We report on the characterization of a time-domain non-contact scanning system for tissue imaging at small source-detector separation based on a supercontinuum laser with acousto-optic tunable filter and time-correlated single photon counting. Late photons were recorded by a fast-gated single-photon avalanche diode (SPAD) while the complete time-of-flight distribution was obtained by a non-gated SPAD in a second channel.

Several phantom measurements were performed according to the nEUROPt protocol in order to characterize spatial resolution and sensitivity of the method.

Depth-dependent contrast and contrast-to-noise ratio as well as lateral resolution were obtained by recording images of small black cylinders of various sizes immersed in a scattering and absorbing liquid at various depths. The analysis was performed for various time windows in both detection channels. This measurement was complemented by simulations obtained with a model that could account for the effects of highly absorbing inclusions and of the measured instrument response function.

In addition, a solid phantom was investigated in which rods of different absorption were inserted at various positions.

The effect of varying distance between tissue and scan head was studied with a tilted solid homogeneous phantom. The varying distance was taken into account by shifting the time windows analysed.

Finally, the responsivity of the detection system, i.e. the ratio of photon count rate detected and input radiance, was measured with a solid slab phantom with known diffuse transmittance factor and nearly Lambertian angular characteristics. The results were compared with those obtained with other instruments for time-domain brain imaging.

8945-19, Session PSun

The optimization of laser parameters in artificial optical cochlear

Yang Liu, Sasa Zhang, Shuo Jiang, Shandong Univ. (China); jun chang, jinbao xia, Shandong University (China)

This paper determined the optimal wavelength range of the laser-induced by measuring the spectral characteristics of cochlear tissues and auditory brainstem response (ABR) in the semiconductor laser system. And we studied the other laser parameters through experimental analysis, such as the optimal frequency, pulse width, laser power, stimulation time and so on.

We can use the cochlear of guinea pig as the research object in the study of laser direct trigger auditory mechanism. Use broadband spectrum light irradiation cochlear in different neural tissues, measure the spectral transmittance characteristics through the microscopic optical fiber spectrometer. Then we explore the influence of laser parameters changes. By the experimental analysis, laser with wavelength between 500nm to 700nm is preferable.

Steps of testing the ABR of the guinea pig are as follows: Firstly, anesthesia the guinea pig, anatomy the cochlear and then make the optical fiber directed toward the modiolus spiral ganglion cells with a distance of about 200um, The square wave pulse signal generator give off pulse electrical signal to drive the semiconductor laser to emit a pulsed light signal. The optical signal transmitted through the optical fiber to optical fiber probe array stimulates the cochlear nerve. ABR testing instrument can test the ABR waveform after it receives the synchronized electrical signals. Then adjust the parameter of the semiconductor laser, and compare the ABR waveform stimulated by laser with those stimulated by the electrical and sound signal. Then find out the optimum frequency, pulse width, laser power, as well as stimulating time.

Conference 8946:
Optical Elastography and Tissue Biomechanics

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8946 Optical Elastography and Tissue Biomechanics

8946-1, Session 1

Optical coherence elastography techniques for assessing biomechanical properties of tissues and cells *(Invited Paper)*

Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Optical coherence tomography (OCT) is being broadly applied and adopted in numerous areas of clinical medicine and surgery, as well as in the non-destructive evaluation of materials. OCT uniquely provides real-time high-resolution depth-resolved imaging in highly scattering media. Using these imaging capabilities to track static and time-dependent dynamic changes from external or internal mechanical forces, numerous optical coherence elastography (OCE) techniques have been developed to extract and quantify mechanical properties of tissues, cells, and non-biological materials. The evolution of OCT to OCE follows the historical trends in other biomedical imaging and elastography modalities using MRI or ultrasound imaging. OCE, however, offers unprecedented resolutions at the micron-scale, and with phase-sensitive methods, cell and tissue displacements on the nanoscale can be monitored. This presentation will review the evolution of OCE and its many associated techniques for interrogating biological specimens and materials. As applications of OCE continue to expand, it is likely that OCE will significantly complement many OCT applications as a means for extracting not only structural but also mechanical information that can be used diagnostically to assess cells and tissues in states of health and disease.

8946-2, Session 1

3D static and shear wave full-field optical coherence elastography of tissues

Amir Nahas, Institut Langevin (France); Stephane Roux, Ecole Normale Supérieure de Cachan (France); Mickael Tanter, A. Claude Boccara, Institut Langevin (France)

Organ structures, tissues and cells are characterized by their intrinsic mechanical properties. Moreover, the mechanical properties of cells are related to their structure and function: changes in those properties reflect cellular healthy or pathological states. Adding this contrast to morphological images could be a highly valuable help for diagnosis. In this study we present two methods to add the elastographic contrast to Full-Field OCT images and our latest results on biological tissues such as ex vivo rat brain and ex vivo human cancerous breast tissue.

The first method is a static method based on finite element 3D digital image correlation algorithm. We register a volumetric image before and after mechanical solicitation of the sample. From those two sets of images we estimate the 3D strain map inside the sample. With this method we not only have access to relative stiffness information but also to mechanical properties of the samples such as mechanical anisotropy or compressibility.

The second method is a quantitative method based on shear wave imaging. In this method we use ultrasound system to generate shear wave inside the sample and by using an ultrafast FF-OCT system (up to 30 kHz) we record the shear wave propagation at the scale given by the FF-OCT resolution. As the local shear wave speed is directly related the local shear modulus, from the movie of the propagation we quantitatively measure local stiffnesses.

8946-3, Session 1

Optical coherence elastography on excised breast cancer specimens: comparison with OCT and histology

Brendan F. Kennedy, Robert A. McLaughlin, Kelsey M. Kennedy, Alan Tien, Lixin Chin, The Univ. of Western Australia (Australia); Bruce Latham, Royal Perth Hospital (Australia); Christobel M. Saunders, David D. Sampson, The Univ. of Western Australia (Australia)

Breast cancer is the second-leading cause of female cancer-related deaths in the USA. A key issue during breast conserving surgery is ensuring complete removal of the tumor. Currently, accurate, microscopic examination of tumor margins is only available postoperatively. OCE is an emerging microscopic imaging technique that provides images, known as elastograms, based on tissue mechanical properties. As the mechanical properties of breast tumor are distinct from that of healthy breast tissue, OCE may be capable of intraoperatively delineating tumor margins with greater accuracy than currently employed intraoperative techniques.

We have developed a portable, phase-sensitive compression OCE system to assess tissue from breast cancer patients. The system includes several optimizations to function in a clinical environment. Use of a common-path interferometer in the underlying OCT system significantly reduces phase noise from background vibrations. Weighted least squares strain estimation accounts for increased phase noise in areas of low OCT SNR, providing greatly improved strain accuracy. We have imaged 40 specimens from 15 patients with this system, obtaining matching H&E histology in each case. Our results demonstrate changes in tissue mechanical properties on a microscopic scale in the presence of infiltrating malignant cells. Areas of malignancy were seen to have different strain to surrounding normal tissue. In addition, the structural disruptions caused by invasive cancer gave rise to a characteristic texture in elastograms. We present results from a number of patients, demonstrating the ability of OCE to visualize these changes. These results confirm that OCE can provide additional contrast to OCT.

8946-4, Session 2

Magnetomotive optical coherence elastography for micro-rheology of biological tissues and cells

Vasilica Crecea, Adeel Ahmad, Benedikt W. Graf, Stephen A. Boppart M.D., Univ. of Illinois at Urbana-Champaign (United States)

Optical coherence elastography (OCE) is an emerging paradigm for measuring the biomechanical properties of tissues noninvasively, in real time, and with high resolution. We present magnetomotive optical coherence elastography (MM-OCE) as a micro-rheology technique for making simultaneous measurements of multiple biomechanical parameters of biological tissues, as well as for probing the biomechanical properties of single cells in culture. Our approach extends the previous contrast-enhancing capabilities of MM-OCT and in a similar manner, utilizes iron oxide magnetic nano or micro particles distributed and embedded in the tissues or cells to serve as transducers for inducing motion. Step-wise or sinusoidal application of an external magnetic field results in displacements in the tissue or cell specimens that are deduced from sensitive amplitude and phase measurements made with the spectral-domain MM-OCE system. In this study, we analyzed freshly

excised rabbit lung and muscle tissues, as well as murine macrophages and human breast cancer cells. We observed that rabbit lung and muscle tissue displacements display characteristic differentiating features in the initial displacement, the damped oscillations, and the creep. The phase-resolved displacements of magnetic microspheres and microbeads from single cells show unique features related to their cellular biomechanics. This MM-OCE micro-rheology approach has the potential to become a novel way of performing real time measurements of biomechanical properties of tissues and cells, and show potential for the development of a new diagnostic and monitoring tool in biology and medicine.

8946-5, Session 2

Quantitative compression optical coherence elastography enabled by a high-resolution stress sensor

Kelsey M. Kennedy, Lixin Chin, Robert A. McLaughlin, David D. Sampson, Brendan F. Kennedy, The Univ. of Western Australia (Australia)

Compression optical coherence elastography (OCE) provides images (elastograms) of tissue strain with high spatial resolution (10s to 100s ?m) and micro-strain sensitivity. Strain elastograms provide relative mechanical contrast between tissues, and have potential to differentiate healthy and diseased areas within a sample. However, absolute measurements of tissue elasticity (Young's modulus) are essential to enable inter-sample comparison and longitudinal studies using OCE. Such measurements require knowledge of both the local strain and local stress at each location in the sample. However, a method for measuring local stress with high resolution has yet to be demonstrated in OCE.

To enable the first quantitative compression OCE measurements, we present a novel stress sensor consisting of a compliant silicone layer placed between the compressor and sample. The strain at each lateral position in the compliant silicone layer is measured using phase-sensitive OCT, and strain elastograms of the sample are simultaneously acquired. The stress-strain behavior of the silicone is well characterized, allowing computation of local stress applied at each location on the sample surface. These stress values are combined with local measurements of strain in the sample to provide absolute estimates of Young's modulus at the sample surface, which are mapped into a quantitative en face elastogram. We present quantitative elastograms of tissue-mimicking phantoms and biological tissue, and compare Young's modulus measurements to those measured using standard bulk compression tests. Results demonstrate the potential for quantitative elastograms to offer improved contrast over standard relative strain elastograms for particular sample geometries.

8946-6, Session 2

Visualization of ultrasonically induced shear wave propagation using phase sensitive optical coherence tomography

Thu-Mai Nguyen, Univ. of Washington (United States); Shaozhen Song, Univ. of Washington (United States) and Univ. of Dundee (United Kingdom); Bastien Arnal, Emily Y. Wong, Matthew O'Donnell, Ruikang K. Wang, Univ. of Washington (United States)

Shear wave elastography measures the stiffness of soft tissues from the speed of a shear wave propagating through tissue. Optical coherence tomography (OCT) is a promising detection modality given its high sensitivity and spatial resolution, making it suitable for elastic characterization of ocular tissues. For clinical applications, it would be valuable to use a non-contact shear source. Thus, we propose acoustic radiation force as a remote shear source combined with OCT for visualization.

A single-element focused transducer (central frequency 7.5 MHz) was used to apply a maximal pressure of ~1 MPa for 100 microseconds in agar phantoms. The subsequent medium relaxation generates a shear wave propagating transversally to the ultrasound beam. Phase-sensitive OCT was used to track shear waves at an equivalent frame rate of 47 kHz. The lateral and axial resolutions are respectively 23 and 5 microns. The local shear wave speed was estimated using a time-of-flight algorithm.

We detected axial displacements with a few hundred nanometers amplitude and a spectrum ranging from 100 Hz to a few kHz. As expected, the shear modulus in a 0.5% agar phantom was estimated to be 5.1 ± 0.5 kPa.

We demonstrated the feasibility of combining acoustic radiation force and OCT to provide a high-resolution, non-contact dynamic elastography method with great potential for ophthalmic elastography. Further studies will aim for in vivo implementation.

8946-7, Session 2

Visualization of shear wave propagation in cornea using optical coherence elastography

Shang Wang, Michael D. Twa, Kirill V. Larin, Univ. of Houston (United States)

We report a noncontact optical method to image the corneal elasticity through reconstructing the shear wave propagation in cornea with 25 kHz frame rate. A focused air-puff system is used to induce the localized and low-amplitude (micron-level) deformation in cornea with short-duration air stream and a phase-sensitive optical coherence tomography (PhS-OCT) system is utilized to detect the corneal surface displacement with high sensitivity. During the data acquisition, the air-puff excitation is maintained at the same position on the surface of cornea, and the OCT beam is set to form a 1-D line scan across the excitation point. Within the scanning line, M-mode OCT imaging is performed at each scanning position, triggered by the signal from the air-puff excitation. With reconstruction from the 3-D matrix of data, this triggering and imaging approach allows the 2-D depth-resolved visualization of the corneal deformation at the rate of the A-line acquisition speed. Using the phase information from the interferometry, we provide the 2-D mapping of the deformation amplitude over time, which shows the guided propagation of the shear wave in cornea. The localized group velocity of the shear wave is quantified based on the curved propagation directions within the cornea. Applying the relationship between the elastic modulus and the shear wave velocity, the quantitative elasticity can be mapped to the cornea with high spatial resolution. Our pilot experiments were conducted on ex vivo eyeballs from young and mature rabbits. With further development, this method is potentially useful for depth-resolved quantification of tissue elastic properties.

8946-8, Session 3

Corneal biomechanical properties from air-puff corneal deformation imaging *(Invited Paper)*

Susana Marcos, Consejo Superior de Investigaciones Científicas (Spain); Sabine Kling, Consejo Superior de Investigaciones Científicas (Spain) and Université de Geneve (Switzerland); Nandor Bekesi, Carlos Dorronsoro, Consejo Superior de Investigaciones Científicas (Spain)

Corneal biomechanics are key for diagnosing corneal pathologies and for evaluating treatments that alter corneal geometry or stiffness. Most methods to measure corneal biomechanics are destructive. Although biomechanical information from strip and flap extensiometry and corneal/whole globe inflation is valuable for testing corneal mechanical models

and in experimental research of disease (i.e. keratoconus) and treatments (i.e. corneal cross-linking), only in vivo techniques will allow using this information for diagnostic purposes and to predict the corneal response to treatment. We present in vivo corneal deformation measurements, obtained by combination of air-puff (as in tonometry systems) with high-speed image acquisition (OCT and Scheimpflug), the contribution of multiple factors (such as intraocular pressure or the presence of the sclera) to the measured deformation, and finite element modeling to retrieve corneal biomechanical properties (elasticity and viscoelasticity) from these measurements

8946-9, Session 3

Air induced deformations: a tool for analysis of nonstructural properties of anterior segment of the human eye

Karol Karnowski, Maciej Wojtkowski, Nicolaus Copernicus Univ. (Poland)

Air induced deformations of anterior segment structures can be directly measured with OCT technique. For this study a ssOCT system combined with air-puff chamber was used. As a light source a commercial available swept source laser working at 50 kHz with a 100 nm optical bandwidth was used (AXSUN TECHNOLOGIES 1300nm). The axial resolution of the system was 9 um in air and the eye was illuminated with average 2.5 mW optical power. Imaging beam goes through the chamber and is coaxially aligned with the exit pipe that delivers air puff. During main measuring protocol a M-scan was recorder. The displacement of corneal surfaces and anterior lens surface was visible. Automatic segmentation procedure detects surfaces positions during deformation and afterwards correction of refraction is applied. The relative displacement of anterior corneal surface, posterior corneal surface and anterior lens surface during the ~20 ms applanation/recovery process can be plotted. Deformation amplitude for corneal anterior and posterior surfaces changes with IOP. Simultaneously any change in corneal biomechanical properties with manifest on deformation readout. Basing only on deformation plots it is impossible to distinguish between influence of IOP or corneal biomechanics. Additional information about the factor that forces deformation allows to plot hysteresis curves. Detailed analysis on anterior segment properties based on such curves is presented here. Set of well control experiments as well as studies performed on healthy volunteers are discussed.

8946-10, Session 3

Elastography methods applicable to the eye

Altaf A. Khan, Soledad Cortina, Univ. of Illinois at Chicago (United States); Wallace Chamon, Univ. of Illinois at Chicago (United States) and Univ. Federal de São Paulo (Brazil); Thomas J. Royston, Univ. of Illinois at Chicago (United States)

Elastography is the mapping of tissues and cells by their respective mechanical properties, such as elasticity and viscosity. Our interest primarily lies in the human eye. Combining Scanning Laser Doppler Vibrometry (SLDV) with geometrically focused mechanical vibratory excitations of the cornea, it is possible to reconstruct these mechanical properties of the cornea. Experiments were conducted on phantom corneas as well as excised donor human corneas to test feasibility and derive a method of modeling. Finite element analysis was used to recreate the phantom studies and corroborate with the experimental data. Results are in close agreement. To further expand the study, lamb eyes were used in MR Elastography studies. 3D wave reconstruction was created and elastography maps were obtained. With MR Elastography, it would be possible to noninvasively measure mechanical properties of anatomical features not visible to SLDV, such as the lens and retina. Future plans include creating a more robust finite element model, improve the SLDV method for in-vivo application, and continuing experiments with MR Elastography.

8946-11, Session 3

Corneal biomechanics with Brillouin microscopy

Giuliano Scarcelli, Sebastien Besner, Seok Hyun Andy Yun, Harvard Medical School (United States)

The mechanical balance between intraocular pressure and corneal stiffness is essential for corneal function. If corneal tissue becomes abnormally weak, corneal ectasia (i.e. thinning and bulging) ensues, causing severe vision degradation. Abnormal weakening of the cornea occurs due to degenerative ocular conditions, e.g. keratoconus, affecting ~1/1000 of the general population or as a complication of LASIK surgery. Concerns about post-LASIK ectasia prevent about 15% of prospective patients from benefitting from laser vision correction. When the clinical symptoms manifest, corneal ectasia is often at an advanced stage that leads to corneal transplant. If corneal weakness were detected early, corneal collagen crosslinking (CXL), a novel therapy under development, has the potential to stop the degenerative bulging; moreover, with mechanical measurements, at-risk subjects could be screened to avoid LASIK surgery. Here, we will show the application of Brillouin microscopy to this clinical need. Brillouin microscopy allows imaging and quantifying changes of elastic modulus of tissue, without contact with high spatial 3D resolution. By providing elasticity-based rather than morphology-based information about the corneal tissue, Brillouin microscopy is sensitive to the mechanical degradation occurring during keratoconus and can characterize the mechanical outcome of CXL. Comparing different protocols of CXL, we demonstrate how this technique may be useful in assessing, in a quantitative manner, the clinical outcomes of CXL procedures as well as comparing different protocols and CXL agents. This work paves the way towards the diagnosis of corneal ectasia and the monitoring of its therapy based on elastic parameters in clinical and in experimental settings.

8946-12, Session 4

GPU-accelerated video-rate optical coherence elastography

Rodney W. Kirk, Brendan F. Kennedy, Lixin Chin, Kelsey M. Kennedy, David D. Sampson, Robert A. McLaughlin, The Univ. of Western Australia (Australia)

We present the first system to provide video-rate optical coherence elastography (OCE) images using a highly parallel implementation based on a graphical processing unit (GPU).

Compression optical coherence elastography (OCE) calculates images (elastograms) of the local mechanical strain of tissue. Generation of elastograms causes significant delays in scanning due to the computationally intensive nature of the calculations. Using a phase-sensitive technique, the spectral data undergoes fast Fourier transformation and the complex result is used to create phase difference images, quantifying tissue displacement. Local strain is given by the rate of change of displacement with depth. Dynamic range and signal-to-noise ratio is improved by applying phase unwrapping and weighted least squares strain estimation. These steps are prohibitively time consuming, limiting previous systems to offline processing, reducing their clinical utility. Highly accelerated processing can be achieved with GPU-based architectures.

GPUs are highly-parallel processing devices, typically based on multiple stream processors, enabling single instruction, multiple data operations. With multiple stream processors, GPUs can process thousands of data elements simultaneously, supporting highly accelerated elastogram generation.

We have developed a system that uses a GPU to produce and display elastograms at video-rates (21 elastograms/second, elastogram size 1000?960 pixels). This enables interactive use of OCE to rapidly generate images to differentiate tissue. The architecture created for this system has a generalised GPU framework to support other processing algorithms. Having validated the system with silicone phantoms of known mechanical properties, we demonstrate the system's capabilities, imaging distinct tissue types in ex vivo biological tissue.

8946-13, Session 4

Simulation and optimization of shear wave detection by laser speckle contrast analysis for cm-depth elasticity imaging

Sinan Li, Cheng Yi, Imperial College London (United Kingdom); Robert J. Eckersley, King's College London (United Kingdom); Daniel S. Elson, Mengxing Tang, Imperial College London (United Kingdom)

Background and Aims:

Shear wave speed is quantitatively related to tissue elastic modulus. Previously we reported an opto-elastography system that tracks shear wave propagation at cm depth in tissue mimicking phantoms using laser speckle contrast analysis [1]. In this work, we describe the theory and develop a simulation tool to optimize the system.

Methods:

Shear wave propagation modulates photon phase and alters the statistics of speckle patterns. In simulation, photon trajectories are predicted by Monte Carlo modeling [2] and shear wave displacement is computed based on the solution given in [3]. The speckle statistics is obtained by calculating the temporal auto-correlation function of light. In experiment, tissue mimicking phantoms (0.8%-1.2% agar and 4% intralipid) are exposed to 532 nm continuous laser and the speckle patterns are received on a CCD camera. Shear waves are generated by transient acoustic radiation force at 1.2 cm depth and a time-resolved CCD contrast difference curve, $\Delta C(t)$, is produced to indicate the shear wave propagation. To measure the local shear wave speed, shear waves are generated at two locations with a different distance relative to the laser axis. The averaged shear wave speed in the differential distance is estimated from the time shift in $\Delta C(t)$.

Results:

The simulated speckle contrast signal due to shear waves matches well with the experimental observations. The results suggest that the shear wave speed and elasticity measurement by dual shear wave interaction signal is more accurate than that with individual shear waves.

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8946-14, Session 4

Laser speckle tracking for monitoring and analysis of retinal photocoagulation

Eric Seifert, Katharina Bliedtner, Ralf Brinkmann, Medizinisches Laserzentrum Lübeck GmbH (Germany)

Laser coagulation of the retina is an established treatment for several retinal diseases. The laser energy and thus the induced thermal damage varies with the transmittance and scattering properties of the anterior eye media and with the pigmentation of the fundus. The temperature plays the most important role in the coagulation process. An established approach to measure a mean depth weighted retinal temperature rise with optoacoustics is demonstrated and reliable but only provides limited information on the coagulation. Phase sensitive OCT potentially offers a three dimensional temporally resolved temperature distribution but is very sensitive to slightest movements which are clinically hard to avoid. We have developed an optical technique able to quantify thermally and coagulation induced tissue movements by dynamic laser speckle analysis (LSA) offering a 2D map of the affected area.

A frequency doubled Nd:YAG laser is used for photocoagulation. Enucleated porcine eyes were used as targets. The spot is 200µm. A Helium Neon laser is used for illumination. The backscattered Helium Neon laser light is captured with a camera and the speckle pattern is analyzed. A Q-switched Nd:YAG is used for simultaneous temperature measurements with the optoacoustic approach. Lesions below ophthalmoscopic visibility have been detected. Radial tissue movements in the micrometer regime have been observed (around 5µm to 20µm in the linear regime between increased laser irradiation/temperature and traveled distance of a speckle). Lesions below ophthalmoscopic visibility have been detected.

8946-15, Session 4

Ultrasound visualization of internal crystalline lens deformation using laser-induced microbubbles

Andrei Karpiouk, Salavat Aglyamov, The Univ. of Texas at Austin (United States); Adrian Glasser, Univ. of Houston (United States); Stanislav Emelianov, The Univ. of Texas at Austin (United States)

The progressive loss of accommodation of the eye, called presbyopia, is a complex and incompletely understood age-related problem. Accommodation in a young eye occurs through a decrease in circumferential diameter of the lens resulting in an increase in surface curvature. Such changes are caused ciliary muscle contraction and through the elasticity of the lens and capsule. Presbyopia results from an increase in stiffness of the lens. One proposed approach to treat presbyopia is to use fs-laser photodisruption of the lens in an effort to improve lens flexibility. However, the elasticity of the crystalline lens increases non-uniformly with age with the nucleus stiffness increasing faster than that of the cortex. Therefore, the deformation within the crystalline lens during accommodation is different in various parts of the lens and likely changes with increasing age.

In this study, ultrasound visualization of microbubbles during deformation in gelatin phantoms and in vitro animal crystalline lenses has been performed. Different patterns of laser-induced microbubbles were created into gelatin phantoms and animal crystalline lenses using a pulsed laser operated at 532 nm with pulse duration of 7 ns.

The phantoms and lenses were then mechanically deformed up to 10% while changes of the microbubbles locations were monitored using high-resolution ultrasound imaging (from 15 to 50 MHz). Displacements of the microbubbles were found using a speckle-tracking correlation algorithm. This approach enables visualization of localized, regional deformation of crystalline lenses and can help to understand the mechanisms of accommodation and presbyopia, improving diagnostics, and potentially developing laser-based presbyopia treatments.

8946-16, Session 4

Wideband optical elastography of in vivo human skin using geometrically focused surface waves

Steven P. Kearney, Zoujun Dai, Thomas J. Royston, Univ. of Illinois at Chicago (United States)

Viscoelastic models are fit to geometrically focused surface (GFS) waves on human skin. Unlike in previous studies on the analytical solution and experimental measurement of radially outward traveling surface waves [Royston et al., J. Acoust. Soc. Am. 106, 3678–3686 (1999)] and [Royston and Dai Z., J. Acoust. Soc. Am. 130 (6), 2011], measurable radially inward traveling GFS waves can be generated over a wider range of frequencies as attenuation is countered by the converging nature of the wavefront. This enables a more accurate and broader assessment of both the shear storage and loss moduli of the material, which are expected to vary with frequency. In the present study, GFS waves are applied to human skin on the posterior side of the forearm using a scanning LASER Doppler vibrometer (SLDV). Surface wave measurements can then be used to estimate the complex frequency dependent viscoelastic properties of biological tissue, which are affected by numerous pathologies. Such measurements on a biological or phantom material can also be used to calibrate measurement of shear viscoelastic properties using other elastographic techniques, such as those based on Doppler ultrasound and magnetic resonance imaging. [Work supported by NIH: Grant # EB012142.]

8946-17, Session 4

Evaluation of fingerprint deformation using optical coherence tomography

Henrique S. Gutierrez da Costa, Stanford Univ. (United States) and Univ. Federal do Paraná (Brazil); Luciano Silva, Univ. Federal do Paraná (Brazil); Audrey K. Ellerbee, Stanford Univ. (United States)

Biometric identification systems have important applications to privacy and security. The most widely used of these, print identification, is based on imaging patterns present in the fingers, hands and feet that are formed by the ridges, valleys and pores of the skin. Most modern print sensors acquire images of the finger when pressed against a sensor surface. Unfortunately, this pressure may result in deformations, which are characterized by changes in the sizes and relative distances of the print patterns, and such changes have been shown to negatively affect the performance of fingerprint identification algorithms.

Optical coherence tomography (OCT) is a novel imaging technique that is capable of imaging the subsurface of biological tissue. Hence, OCT may be used to obtain images of subdermal skin structures from which one can extract an internal fingerprint. The internal fingerprint is very similar in structure to the commonly used external fingerprint and is of increasing interest in investigations of identify fraud.

The elasticity of the biological tissue located between the stratum corneum and the dermis was hypothesized to play a role in dampening the pressure associated with finger-sensor contact. If true, one would expect the internal fingerprint to be less deformable overall and, therefore, capable of yielding a more reliable print from the perspective of security. We used OCT to measure the distributed pressures applied to a transparent surface while scanning the fingerprints of human volunteers. We present metrics to characterize print deformation and correlate them with differences in skin biomechanics.

8946-18, Session 5

New light on cell manipulation and rheology *(Invited Paper)*

Kishan Dholakia, Univ. of St. Andrews (United Kingdom)

This talk will describe recent work on cell manipulation and transfection at St Andrews. Topics will include new ways for transfection using femtosecond lasers and laser induced particle breakdown. this includes a range of cells including primary neurons, with relevance for optogenetics. Using stroboscopic quantitative phase microscopy we study cell deformation and the response to cavitation bubbles and transient shear stress. Finally we explore cell microrheology and membrane dynamics of cells, including transfected cells using controlled rotation of trapped particles

8946-19, Session 5

Ultra-fast optical manipulation of single proteins binding to the actin cytoskeleton

Marco Capitanio, Univ. degli Studi di Firenze (Italy) and European Lab. for Non-linear Spectroscopy (Italy); Lucia Gardini, European Lab. for Non-linear Spectroscopy (Italy); Francesco S. Pavone, European Lab. for Non-linear Spectroscopy (Italy) and Univ. degli Studi di Firenze (Italy) and National Institute of Optics (Italy)

In the last decade, forces and mechanical stresses acting on biological systems are emerging as regulatory factors essential for cell life. In particular, mechanical load is continuously exerted by intracellular proteins and molecular motors to the cell's cytoskeleton, and determine the shape and function of cells. The conversion of these mechanical forces into biochemical and biomolecular signals is at the base of many biological processes fundamental for the development and differentiation of cells, for their correct function and for the development of pathologies.

We recently developed an in vitro system that allows the investigation, at the single molecule level, of the interaction of proteins binding the actin cytoskeleton, and how such interaction is modulated by external forces. Our system displays a delay of only ?10 ?s between formation of the molecular bond and application of the force and is capable of detecting interactions as short as 100 ?s. The force-clamp configuration in which our assay operates allows direct measurements of load-dependence of lifetimes of single molecular bonds. Moreover, conformational changes of single proteins and molecular motors can be recorded with sub-nanometer accuracy and few tens of microseconds of temporal resolution.

8946-20, Session 5

Dark-field Brillouin microscopy for elasticity imaging

Giuseppe Antonacci, Imperial College London (United Kingdom); Matthew R. Foreman, Max-Planck-Institut für die Physik des Lichts (Germany) and Imperial College London (United Kingdom); Carl Paterson, Peter Török, Imperial College London (United Kingdom)

Spontaneous Brillouin scattering is an inelastic scattering process arising from inherent thermal density fluctuations, or acoustic phonons, propagating in a medium. Over the last few years, Brillouin spectroscopy has shown great potential to become a reliable non-invasive diagnostic tool due to its unique capability of retrieving viscoelastic properties of materials such as strain and stiffness.

The detection of the weak scattered light, in addition to the resolution of the Brillouin peaks (typically shifted by few GHz from the central peak) represent one of the greatest challenges in Brillouin. The recent development of high sensitivity CCD cameras has brought Brillouin spectroscopy from a point sampling technique to a new imaging modality. Furthermore, the application of Virtually Imaged Phased Array (VIPA) etalons has dramatically reduced insertion loss simultaneously allowing fast (<1s) collection of the entire spectrum.

We describe the characterisation of a new dark-field confocal Brillouin microscope designed to measure elasticity properties of biological tissues. Such information can be used, for example, for diagnosis of diseases such as cancer. Brillouin spectra of a variety of liquids have been obtained in dark-field mode using a custom built spectrometer. Furthermore, the frequency broadening of the Brillouin spectrum due to finite illumination and collection apertures has been investigated in order

to determine the optimal geometry that maximises both the spectral and optical resolution. Experimental results confirm a narrower Brillouin peak in backscattering configuration enabling the employment of high NA microscope objectives. Preliminary Brillouin images of different solids and liquids interfaces are acquired.

8946-21, Session 5

Linking cell shape, elasticity and fate:: in vitro re-differentiation of chondrocytes

Xiaofei Yuan, Univ. of Glasgow (United Kingdom); Yahua Chim, University of Glasgow (United Kingdom) and University of Glasgow (United Kingdom); Huabing Yin, Univ. of Glasgow (United Kingdom)

Cells adapt their shape in response to their extracellular matrix (ECM). Many phenomena show that cell shape is a potent regulator of cell growth, physiology and differentiation. The use of engineered ECM to control cell shape for desirable cellular function has shown great potential in tissue engineering and regenerative medicine. However, little is known about how cell shape affects the biomechanical properties of cells and subsequent cell function.

In this study we employed a combination of microfabrication, Atomic Force Microscopy (AFM) and immunostaining to investigate potential links between these three factors. We have designed and fabricated a series of 2D and 3D micropatterns that mimick geometric confinements of single cells (e.g chondrocytes) found in vivo, with the aim of regulating differentiation of primary chondrocytes and Mesenchyme Stem Cells (hMSC). We have formulated a rigorous approach to quantify cellular elasticity using AFM via systematic evaluation of operative factors and mathematical models for data fitting. It was found that cell differentiation and elasticity are different between the cells restricted within the patterns and those unrestricted. Furthermore, the size of patterns also showed a significant role. This approach will provide valuable information that will enhance our ability to better engineer artificial ECMs that guide cell differentiation for targeted applications.

8946-22, Session 5

Membrane mechanics in erythrocytes infected with transgenic malaria parasites

Poorya Hosseini, Zeinab Aboud, Youngwoon Choi, Peter T. C. So, Zahid Yaqoob, Massachusetts Institute of Technology (United States)

Red Blood Cells (RBCs) have the important task of delivering oxygen from the lungs to the tissues and organs all over the body. The ability of RBCs to deliver oxygen may be compromised in a range of diseases known as blood abnormalities. One such blood abnormality, malaria, is marked by the spread of parasites within the cytosol, causing dramatic changes in morphology, biochemistry and biomechanics of the host cell. Previous studies have investigated changes in the mechanics and biochemistry of RBCs over different stages of malaria infection [1]. Specific genes expressed by the parasite within the host cell environment induce some of these structural changes. The human genome and that of the deadliest of the malarial parasites (Plasmodium falciparum) have been successfully sequenced [2]. However, further investigations are needed to elucidate the link between the P. falciparum genome and the phenotypic changes observed in RBCs, and how these changes affect evolution of the parasite during its life cycle. Using the latest near-common-path quantitative phase microscope developed in our lab [3] combined with fluorescent imaging capability, we are studying these changes and their correlation with the biological changes introduced by two transgenic parasite lines.

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8946-23, Session 5

Non-gaussian dynamics of red blood cell membrane fluctuation using diffraction phase microscopy

Hyunjoo Park, Sangyeon Cho, YongKeun Park, KAIST (Korea, Republic of)

We present athermal dynamics of membrane fluctuation in red blood cell (RBC) using diffraction phase microscopy (DPM). DPM is a quantitative phase imaging technique based on a common-path laser interferometry that measures dynamic motions of biological samples with nanometer sensitivity. The dynamic membrane fluctuations of RBCs, measured by DPM, are systematically analyzed to address non-Gaussian dynamics as a function of local curvature. Since the deformability of RBC is important for blood circulation, especially in micro-capillary, the distinctive discocyte shape of RBCs, which is mainly regulated by ATP, help RBCs to deform and recover. In order to study the dynamics of membrane fluctuation, we control the ATP activity by either depleting or reintroducing it and then evaluated non-Gaussian parameters from height fluctuation spatially and temporally measured from DPM. We show non-Gaussian dynamics is prominent in outer convex and the special spatial frequency peaks are mostly localized in dimple region when ATP is present . However, the level of non-Gaussianity decreases and the distinct frequency peaks also disappear in the absence of ATP. In addition, we employ sickle cells (SC), which of spectrin network may be disrupted by abnormal hemoglobin, to see the effect of cytoskeleton integrity as well as ATP. Despite of discocyte shape in SC, it shows non-Gaussianity for outer convex region is still enhanced in similar to normal RBC, but the distinct spatial frequency peaks are not found. These results lead us to new insight of biochemical model for cell membrane cortex via employing a quantitative phase imaging technique.

8946-24, Session 5

Rate-dependent dynamics of cellular membranes probed by laser tweezers and optical displacement sensing

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Cellular plasma membranes are biological nanostructures containing mainly lipids and protein molecules. Mechanical properties of cellular membranes play important roles in their biological functions as well as the function of the living cell as a whole. Although synthetic membrane systems composed of purified lipids and proteins have revealed important aspects of membrane mechanics, these biomimetic systems lack the complexities involved in living cell membranes arising from the

conformational properties of membrane proteins, spatial arrangements of the lipids and proteins, and the interaction of these constituents with the underlying cytoskeleton.

In this study, we investigated the nanomechanical properties of cell membranes in response to elongation at different rates by an optically-trapped fluorescent microsphere as the pulling probe. Specifically, we pulled nanotubes to 20 μm length, and recorded the subsequent time-resolved force relaxation with a quadrant photodetector-based displacement detection system. A viscoelastic model, consisting of a Kelvin body in parallel with a Maxwell element, was employed to analyze the force dynamics (relaxation) of the membrane nanotubes. The force relaxation response of membrane nanotubes exhibited fast relaxation with a time of 0.388±0.21 s (mean ± s.d.) followed by a much slower relaxation process with time course of 11.74±3.35 s, in response to pulling rate of 1 ?m/s. The membrane nanotubes pulled at higher pulling rates exhibited similar two-stage force relaxation responses. Specifically, the fast and slow relaxation times significantly decreased to 0.104±0.05 s, and 2±1.2 s in response to 100 ?m/s pulling rate, respectively. The present work elucidates the role of membrane nanomechanics in biological processes involving slow and fast deformations of living cells and cellular membranes such as those in the cochlear outer hair cells performing within a broad frequency range from several Hz to tens of KHz.

8946-25, Session 6

Use of phase sensitive OCT to track and visualize dynamic mechanical wave propagation within tissue *(Invited Paper)*

Ruikang K. Wang, Univ. of Washington (United States)

No Abstract Available

8946-26, Session 6

Confocal acoustic radiation force optical coherence elastography

Rui Li, Beckman Laser Institute and Medical Clinic (United States); Wenjuan Qi, Univ. of California, Irvine (United States); Teng Ma, Qifa Zhou, Koping Kirk Shung, The Univ. of Southern California (United States); Zhongping Chen, Beckman Laser Institute and Medical Clinic (United States) and Univ. of California, Irvine (United States)

The mechanical properties of living tissue are critical indicators of tissue pathological conditions as many diseases progress with mechanical alterations. Phase resolved optical coherence elastography (PR-OCE) is a novel elastography technology, which uses optical coherence tomography (OCT) as the in vivo imaging modality to measure tissue biomechanical properties on a scale of micrometers and provide functional information. In this study, we designed a novel confocal acoustic radiation force (ARF) OCE system, which to the best of our knowledge, is the first OCE system equipped with confocal, coregistered ultrasound stimulation and OCT detection. A ring ultrasound transducer was used to generate an oscillatory ARF as an internally localized vibrator to induce motion along the axial directions of the tissue and we detected the tissue motions by measuring the motion-induced phase changes between successive A lines, which were used to generate A-mode phase maps to resolve the instantaneous tissue deformations. Quantitative measurements have been made on phantoms demonstrating the ability of our system to quantitatively map the elastic property of materials. Furthermore, we performed experiments on a section of human coronary artery with atherosclerotic plaques. Both phantom and human tissue tests indicate that this system is able to sense the stiffness difference between samples. Featuring high axial resolution, high speed and high motion sensitivity, our confocal setup promises great potential for point by point elastic imaging in vivo and differentiation of diseased tissue from normal tissue, such as identifying the composition of atherosclerotic lesions, which is of great importance for atherosclerosis diagnosis and treatment.

8946-27, Session 6

Acoustic radiation force optical coherence elastography of phantoms and biological tissues based on focused ultrasound excitation

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Recent developments in optical coherence elastography (OCE) have explored the use of acoustic radiation force for dynamic mechanical loading of samples, utilizing both focused and unfocussed ultrasound beams. In this study we extend the use a focused ultrasound excitation (8 MHz) to perform dynamic OCE in the previously unexplored frequency regime of several kilohertz, up to a maximum of 10 kHz. We present shear wave dispersion curves for tissue phantoms with known shear modulus, and for liver tissue undergoing formalin fixation. The liver sample undergoing fixation (shear modulus increasing with time) showed significant differences in the dispersion curves over a 2-hour timeframe, whereas the dispersion curves for a control liver sample in saline were highly repeatable over the same time scale. Fitting of these dispersion curves with a Kelvin-Voigt model showed that the shear modulus of the liver undergoing fixation increased from 1.15 kPa to 12.1 kPa over the 2-hour timeframe. The corresponding vibration amplitude decreased from 1.87 um to 0.28 um. In separate experiments on gelatin phantoms, we found that the attenuation coefficient of shear waves at frequencies of several kilohertz was significantly higher than in the hundreds of Hertz range, i.e. the mechanical interaction length at higher frequencies was significantly shorter, potentially reducing the mechanical coupling between nearby regions. These results suggest that excitation in the several kilohertz regime and higher, combined with a confocal alignment of ultrasound and OCT beams, may be advantageous for dynamic OCE that aims to isolate local mechanical properties with high spatial resolution.

8946-28, Session 6

Model-based optical coherence elastography using acoustic radiation force

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Acoustic Radiation Force (ARF) stimulation is actively used in ultrasound elastography to estimate mechanical properties of tissue. Compared with ultrasound imaging, OCT provides advantage in both spatial resolution and signal-to-noise ratio. Therefore, a combination of ARF and OCT technologies can provide a unique opportunity to measure viscoelastic properties of tissue, especially when the use of high intensity radiation pressure is limited for safety reasons.

In this presentation we discuss a newly developed theoretical model of the deformation of a layered viscoelastic medium in response to an acoustic radiation force of short duration. An acoustic impulse was considered as an axisymmetric force generated on the upper surface of

the layer. An analytical solution of this problem was obtained using the Hankel transform in frequency domain. It was demonstrated that layers at different depths introduce different frequency responses.

To verify the developed model, experiments were performed using tissue-simulating, inhomogeneous phantoms of varying mechanical properties. The Young's modulus of the phantoms was varied from 5 to 50 kPa. A single-element focused ultrasound transducer (3.5 MHz) was used to apply the radiation force with various durations on the surface of phantoms. Displacements on the phantom surface were measured using a phase-sensitive OCT at 25 kHz repetition frequency. The experimental results were in good agreement with the modeling results. Therefore, the proposed theoretical model can be used to reconstruct the mechanical properties of tissue based on ARF/OCT measurements.

8946-29, Session 6

Multiphysics simulation of optical coherence elastography images using combined optical and mechanical models

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We present a model of image formation in optical coherence elastography (OCE) that is capable of producing realistic simulated images (elastograms). We achieve this by simulating both the mechanical deformation of the sample and the imaging modality, optical coherence tomography (OCT), used to estimate tissue displacement. This multiphysics model will enable us to explore fundamental properties of OCE and to better interpret experimentally obtained elastograms.

Using the finite element method (FEM), we compute the deformation of the sample in response to an applied mechanical load, and calculate the resulting displacement at each point in the sample. The acquisition of the corresponding OCT images is then simulated using an optical model based on the extended Huygens-Fresnel formulation of beam propagation, including the effects of speckle, noise and attenuation. Corresponding elastograms are generated using the simulated OCT images of the sample before and after mechanical loading.

We demonstrate the ability of this model to simulate strain elastograms generated by phase-sensitive compression OCE. Samples are deformed using quasi-static compressive loading, local displacement is calculated from the phase difference between OCT B-scans acquired before and after the applied load, and elastograms are generated from the rate of change of sample displacement with depth. The results of our multiphysics model of OCE are validated against experimental data of structured phantoms of varying geometries and mechanical properties. The framework we present will be important both in future comparisons of OCE techniques and in determining the efficacy both of new loading mechanisms and displacement estimation techniques.

8946-30, Session 7

Imaging the cellular response to transient shear stress using time-resolved digital holography

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Shear stress has been recognized as one of the biophysical methods by which to permeabilize plasma membranes of cells. In particular, high pressure transient hydrodynamic flows created by laser-induced cavitation have been shown to lead to the uptake of fluorophores and plasmid DNA. While the mechanism and dynamics of cavitation have been extensively studied using a variety of time-resolved imaging techniques, the cellular response to the cavitation bubble and cavitation induced transient hydrodynamic flows has never been shown in detail. We use time-resolved quantitative phase microscopy to study cellular response to laser-induced cavitation bubbles. Laser-induced breakdown of an optically trapped polystyrene nanoparticle (500nm in diameter) irradiated with a single nanosecond laser pulse at 532nm creates transient shear stress to surrounding cells without causing cell lysis. A bi-directional transient displacement of cytoplasm is observed during expansion and collapse of the cavitation bubble. In some cases, cell deformation is only observable at the microsecond time scale without any permanent change in cell shape or optical thickness. On a time scale of seconds, the cellular response to shear stress and cytoplasm deformation typically leads to retraction of the cellular edge most exposed to the flow, rounding of the cell body and, in some cases, loss of cellular dry mass. These results give a new insight into the cellular response to laser-induced shear stress and related plasma membrane permeabilization. This study also demonstrates that laser-induced breakdown of an optically trapped nanoparticle offers localized cavitation (70 microns in diameter), which interacts with a single cell.

8946-31, Session 7

Dimensional characterisation of collagen constructs in situ

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We present results of a non contacting instrument based on the confocal scanning technique for assessing the thickness and structure of collagen substrates and tissue constructs. There is an unmet need in the creation of tissue constructs to qualitatively evaluate their dimensional characteristics during manufacture. With this knowledge more effective structures can be produced.

The measurement is complicated by the need to make these measurements in situ. For many processes, including the RAFT (Real Architecture for 3D Tissue) process for generating 3D structures, the constructs are situated in a liquid solution contained in a well plate or similar container. It is therefore necessary to perform the measurements through an interfering medium and this confounds many measurement techniques.

A system has therefore been developed that utilises a scanning confocal arrangement to accurately measure the dimensional characteristics of these constructs in situ. A fibre based optical arrangement using compact, proven components from the telecommunications industry is integrated into a dedicated system architecture so that the constructs can be measured in production. This architecture is particular important due to the "wet" nature of the samples. The meter can measure constructs with thicknesses from a few tens of micrometres up to about a millimetre and has sub-micrometre resolution.

Results will be presented that show how the meter has been used to evaluate changes in the constructs whilst in production. This was little understood prior to these measurements and the greater understanding of how the materials behave has allowed the process to be greatly improved.

8946-32, Session 7

Optical rheology of blood coagulation

Zeinab Hajjarian Kashany, Seemantini K. Nadkarni, Markandey M. Tripathi, Harvard Medical School (United States)

Technologies that evaluate the whole blood viscoelasticity during the clot formation in real-time provide significant insight into coagulation defects in patients. Currently available coagulation assessment techniques are contact-based and evaluate blood mechanical properties by stirring the blood using a rod in a cup, which makes the blood specimen susceptible to shear thinning and strain hardening. We describe the development of Optical Thromboelastography (OTEG), a novel approach to enable mechanical evaluation of coagulating blood in a non-contact manner. In OTEG, a few drops of blood are illuminated by a laser beam and temporally fluctuating speckle patterns, scattered by intrinsic scattering particles, are recorded by a high speed camera for alternating intervals during the course of coagulation. Due to progressive stiffening of the blood sample during the formation of a platelet-fibrin clot, mean square displacements (MSD) of scattering particles are gradually reduced and speckle intensity fluctuations slow down accordingly. Speckle frame series are processed to evaluate the speckle intensity temporal autocorrelation function, g2(t), from which the MSD of scattering particles is extracted. The MSD is replaced in the generalized Stokes-Einstein relation (GSER) and a quantity proportional to the viscolastic modulus G*(?) is deduced. Concurrent to LSR measurements, rotational rheology is performed on a 2ml of blood from the same donor in a time sweep procedure. Results of linear regression analysis demonstrate a statistically significant strong correlation between OTEG and standard mechanical rheometry (R=0.96, P< 0.0001). These results highlight the significant potential of OTEG in evaluating the viscoelasticity of clotting blood in a non-contact, non-destructive manner.

8946-33, Session 7

Evaluation of blood coagulation parameters using optical thromboelastography (OTEG)

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Bleeding disorders are often acquired due to impaired coagulation following fluid resuscitation or prolonged anticoagulant use in trauma and surgical patients. Large volume administration of crystalloids lowers fibrinogen levels impairing fibrin polymerization, and anticoagulant administration prolongs clotting time with slower clot progression rate. Optical thromboelastography (OTEG), a new technique to evaluate blood coagulation status, has the potential to advance clinical capability to tailor fluid resuscitation and anticoagulant administration in patients at risk of bleeding disorders. The goal of this study is to test the accuracy of OTEG in measuring blood coagulation status following hemodilution with lactated Ringer's solution (LR) and addition of heparin anticoagulant. In OTEG, temporal speckle intensity fluctuations are analyzed using a CMOS camera, and changes in the viscoelastic properties of clotting blood are measured to evaluate clotting time (R), clot progression rate (?), and maximum clot stiffness (MA). In this study, varying concentrations of LR and heparin are mixed with swine whole blood, and alterations in MA, R-time and ? caused by hemodilution and anti-coagulation are measured using OTEG. Our results show that MA reduces from 60% to 13% for 40-70% LR dilution, and heparin addition (0.5USP/ml) increases R-time from 4.2 to 12.6 minutes while lowering ? from 730 to 300. In addition, OTEG results show a strong correlation with standard-reference mechanical Thromboelastography (TEG) for MA (R=0.98, p<0.01), R-time (R=0.96, p<0.01) and ? (R=0.97, p<0.01). These results demonstrate that OTEG can accurately evaluate changes in blood coagulation status secondary to hemodilution and anti-coagulation.

8946-34, Session 7

Rapid assessment of elevated level of protein content in meningitis using elasticity measurements

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Brillouin spectroscopy is an established technique for material characterization and elastic constant determination. The apparent renaissance of Brillouin spectroscopy has been seen in recent years is attributed to development of better optical instruments, which allow fast non-destructive acquisition of Brillouin spectra.

In this report, we present a novel method of detecting elevated protein levels in the cerebrospinal fluid (CSF) that are characteristic of bacterial and fungal meningitis [1]. This presents a significant step toward a clinical screening which is completely nondestructive to a CSF sample obtained from a typical lumbar puncture.

Bacterial meningitis is of global clinical significance. Immediate treatment with antibiotics is essential to improving the mortality and morbidity associated with the disease when left untreated. However, bacterial meningitis can be a challenging diagnosis, primarily due to the non-specificity of symptoms, particularly in small children. In essentially all cases, however, protein concentrations in the CSF are elevated [2] resulting in a change of elastic properties [3]. With this in mind, we developed and validated a method of detecting elevated protein concentrations in CSF based on Brillouin spectroscopy.

We modified Brillouin spectrometer to accommodate it for imaging microscopic samples in the presence of substantial light scattering and used this newly developed instrument to characterize controlled buffer solutions with a varying concentration of albumin and gamma-globulin. We were able to achieve a sensitivity of detection of about 0.5 mg/mL. Using those results as a guidance, we validated our hypothesis for CSF samples obtained from a local veterinarian school.

References:

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8946-35, Session PSun

Optical coherence tomography detection of shear wave propagation in MCF7 cell modules and carotid artery samples

Marjan Razani, Adrian Mariampillai, Ryerson Univ. (Canada); Tim-Rasmus Kiehl, Univ. Health Network (Canada); Victor Yang, Michael C. Kolios, Ryerson Univ. (Canada)

In this work, we explored the potential of measuring shear wave propagation using Optical Coherence Elastography (OCE) in MCF7 cell modules (comprised of MCF7 cells and collagen) and carotid artery samples. Shear waves were generated using a piezoelectric transducer transmitting sine-wave bursts of 400 ?s and using OCT as an imaging modality to detect the shear wave propagation and measure the mechanical properties of MCF7 cells and carotid artery samples. Acoustic radiation force was applied to the samples that were embedded in a gel. OCT phase maps were acquired with a swept-source OCT (SS-OCT) system. Differential OCT phase maps, measured with and without the acoustic radiation force, demonstrate microscopic displacement generated by shear wave propagation that was detectable in both samples. The structures from the sample histology were spatially correlated to the OCT phase maps. This method lays the foundation for future studies of mechanical property measurements of breast cancer structures and intravascular structure, with applications in the study of breast cancer and vascular pathologies. This method of SW-OCE for investigating tissue mechanical properties via shear wave measurements

will be explored in vitro and in vivo for atherosclerotic vascular tissues for which significant contrast in the shear modulus is expected between the lipid core of an atherosclerotic plaque and the fibrous plaque.

8946-36, Session PSun

An OCT-based air suction-indentation probe for tissue elasticity measurement

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In this study, we developed a miniaturized optical coherence tomography (OCT) probe with a diameter of 4 mm. It was integrated with an air-jet indentation and air suction to induce deformation of tissue. The deforming process of tissue under suction or indentation was continuously monitored by OCT, and deformation of tissue was then derived from the transient OCT signals. Studies on phantoms with different stiffness were conducted. Results showed that the stiffness obtained by the OCT-based suction and indention well correlated with the stiffness detected using conventional mechanical testing. The probe was small enough for endoscopic use. In addition to the elasticity, the viscoelasticity of tissues can also be detected using creep indentation and suction test.

Conference 8947: Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XII

BiOS SPIE Photonics West

Monday - Thursday 3 –6 February 2014

Part of Proceedings of SPIE Vol. 8947 Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XII

8947-1, Session 1

Fluorescence lifetime imaging of NADH and FAD reports therapeutic effects on pancreatic cancer metabolism

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Pancreatic cancer is difficult to eradicate due to a high stromal content, close proximity to vital organs/arteries, and a lack of effective drugs. These difficulties are exacerbated by the short lifespan of patients, demonstrating a need for effective, first round therapeutics. We have developed a novel in vitro model for preclinical and clinical drug testing. By using a tumor/stromal cell co-culture technique, combined with fluorescence lifetime imaging of NADH and FAD, we are able to dynamically image the metabolism of malignant and stromal cells to evaluate drug efficacy. For this study, murine pancreatic tumors are harvested, mechanically separated into 100-300 micrometer organoids, which are embedded into a collagen matrix and overlain with growth media supplemented with traditional and experimental pancreatic cancer drugs. Fluorescence lifetime imaging of NADH and FAD is performed at 24, 48, and 72 hours post treatment. Gemcitabine induces a decrease in the redox ratio (fluorescence intensity of NADH divided by that of FAD), and an increase in the mean NADH and FAD fluorescence lifetime by 48 hr ($p<0.05$). AZD 1480, an experimental drug, likewise induces measurable changes in the optical metabolic endpoints by 24 and 48 hr post drug treatment ($p<0.05$). These results corroborate with immunofluorescence of organoid growth and in vivo tumor growth studies. This platform provides a bridge between preclinical and clinical studies and has potential as a prognostic screen for pancreatic cancer patients.

8947-2, Session 1

Raman microbeam spectrometer noninvasively measures biomoelcules to monitor the tryptophan metabolic pathway

Gregory Michel, Alan Bigelow, Jamie Harden, The Rockefeller Univ. (United States); James G. Krueger M.D., Rockefeller Univ (United States); Daniel S. Gareau, The Rockefeller Univ. (United States)

Efforts must be made to improve early detection of melanoma to increase the accuracy of diagnosis and avoid unnecessary surgical excisions of common moles. We propose noninvasive quantitative spectral fingerprinting of protein expression in lesions using Raman spectroscopy within a confocally gated volume of tissue. L-tryptophan catabolism is upregulated in the tumor micro-environment and inhibits the immune response that usually is tumor suppressive. The tryptophan pathway is therefore worthy of diagnostic measurement and finding the ratio of L-tryptophan to its metabolites may aid a melanoma diagnosis. We report the intensity of the Raman signal from L-tryptophan, L-kynurenine, 3-hydroxyanthranilic acid, and quinolinic acid during different stages of the tryptophan pathway.

8947-3, Session 1

Digital holographic microscopy for monitoring growth and treatment response in 3D in vitro tumor models

Yuyu Li, Ljubica Petrovic, Jonathan P. Celli, Chandra S. Yelleswarapu, Univ. of Massachusetts Boston (United States)

Three-dimensional tumor models, which restore physiologically-relevant 3D tumor architecture and signaling have emerged as valuable tools in cancer research. Yet, in order to utilize these biologically relevant models to their full potential there is need for further development of 3D microscopy tools that allow longitudinal monitoring of growth processes without termination of cultures at the time of imaging. For example laser scanning confocal systems provide the ability to obtain depth resolved optical sections but typically require destructive fixation and staining procedures which necessitate termination of the culture. Other widely available microscopy methods, such as DIC and phase contrast provide only qualitative insight into sample depth. Digital holographic microscopy (DHM) is a non-destructive, full-field quantitative phase imaging technique that can layout the structural details in 3D and is suitable in live cell imaging scenarios. We recorded digital holograms of pancreatic cancer cells, overlaid on Matrigel, for several days. Then the holograms are digital processed and the unwrapped phase images were obtained. By analysis of unwrapped phase measurements we demonstrate the use of DHM for monitoring the growth and development of 3D multicellular pancreatic tumor nodules extracellular matrix overlays subject to chemotherapy or photodynamic therapy (PDT) treatments and under normal growth conditions. This work suggests the utility of DHM as a useful modality to be implemented in conjunction with 3D tumor models and facilitate more widespread implementation of these model systems for longitudinal monitoring of 3D growth processes.

8947-4, Session 1

Optical monitoring of glucose demand and vascular delivery in a preclinical murine model

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Targeted therapies such as PI3K inhibition can affect tumor vasculature, and hence delivery of imaging agents like FDG, while independently modifying intrinsic glucose demand. Therefore, it is important to identify whether perceived changes in glucose uptake are caused by vascular or true metabolic changes. This study sought to develop an optical strategy for simultaneously quantifying tissue glucose uptake and vascular oxygenation (SO2) free of cross-talk.

Glucose uptake was measured using a fluorescent D-glucose derivative 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-deoxy-D-glucose (2-NBDG). 2-NBDLG, an enantiomer of NBDG that is unrecognized by GLUT receptors, served as a non-specific control. Mice implanted with dorsal skin flap window chambers were injected with 2-NBDG (2, 6 or 10 mM; n = 7 mice/dose) or 2-NBDLG (2mM; n = 3 mice) for hyperspectral imaging. SO2 was calculated from wavelength-dependent hemoglobin absorption and NBDG/NBDLG uptake curves were constructed using tissue-space fluorescence intensities.

The time to reach maximum 2-NBDG uptake was dependent on baseline SO2, irrespective of dose ($n = 21$; $p < 0.05$). By 10 minutes after injection,

there was no significant difference in uptake of 2-NBDG and 2-NBDLG (2mM; $p < 0.05$). However, 75 minutes after injection, fluorescence due to 2-NBDG was greater than 2-NBDLG (n=3/group, $p<0.05$). In addition, blood glucose level was correlated with 2-NBDG fluorescence at 60 minutes for the highest dose ($r = -0.877$; $p = 0.01$). In summary, our data indicates that a combination of SO2 and glucose uptake can report on delivery effects at early time points, and report on glucose uptake at later time points.

8947-5, Session 1

Network signatures of nuclear and cytoplasmic density alterations in a model of pre- and post-metastatic colorectal cancer

Dhwanil Damania, Hariharan Subramanian, Vadim Backman, Northwestern Univ. (United States); Eric Anderson, Melissa Wong, Owen J. T. McCarty, Kevin G. Phillips, Oregon Health & Science Univ. (United States)

Cells contributing to the pathogenesis of cancer possess cytoplasmic and nuclear structural alterations that accompany their aberrant genetic, epigenetic, and molecular perturbations. While it is known that architectural changes in primary and metastatic tumor cells can be quantified through cellular density variations at both the nanometer and micrometer spatial scales, the interdependent relationships among nuclear and cytoplasmic density as a function of tumorigenic potential has not been thoroughly investigated. Using the isogenic SW480 and SW620 cell lines as a model of pre- and post-metastatic transition in colorectal cancer, we demonstrate that nuclear and cytoplasmic nano-scale disorder, micron-scale dry mass content, mean dry mass density, and shape metrics of the dry mass density histogram are uniquely correlated within and across different cellular compartments for a given cell type. The correlations of these physical parameters can be interpreted as networks whose nodal importance and level of connection independence differ according to disease stage. This work demonstrates how biophysical parameters are linked within and across different cellular compartments during the architectural orchestration of the metastatic phenotype.

8947-6, Session 1

Endogenous two-photon excited fluorescence microscopy can elucidate metabolic changes in precancerous tissues

Irene Georgakoudi, Antonio Varone, Joanna Xylas, Kyle P. Quinn, Tufts Univ. (United States); Margaret McLaughlin-Drubin, Karl Münger, Brigham and Women's Hospital (United States)

Non-invasive methods capable of providing spatially-resolved metabolic information could advance our understanding of mechanisms that underlie key tissue transformations, that occur during normal and diseased development or healing. In this study, we demonstrate how such methods could be sensitive to subtle metabolic changes in key pathways often perturbed during cancer development. We use nonlinear optical microscopy relying on endogenous keratin, NADH and FAD fluorescence to acquire high-resolution, three-dimensional images of epithelial tissues in which cell signaling pathways are disturbed by the expression of the full human papilloma virus 16 (HPV16) or by the isolated expression of HPV16 oncoproteins, E6 and/or E7. We identify unique depth-dependent optical metabolic profiles for each epithelial tissue type we examine, as assessed by the optical redox ratio defined as FAD/(NADH+FAD). We validate the sensitivity of these optical redox ratio measurements using liquid chromatography / tandem mass spectrometry (LC/MS-MS) and biochemical substrate consumption assays. In combination, these studies suggest that optical metabolic assessments can be sensitive to distinct changes in the dominance of major metabolic pathways such as glycolysis, oxidative phosphorylation and glutaminolysis. Therefore, these optical non-invasive methods could potentially provide important spatiotemporal insights on the role of different metabolic pathways in the development and detection of cancer in particular and of a number of metabolic diseases more broadly.

8947-7, Session 2

In vivo imaging of spinal cord in contusion injury model mice by multiphoton microscopy

Yusuke Oshima, Hideki Horiuchi, Tadanori Ogata, Atsuhiko Hikita, Hiromasa Miura, Takeshi Imamura, Ehime Univ. (Japan)

Fluorescent imaging technique is a promising method and has been developed for in vivo applications in cellular biology. In particular, non-linear optical imaging technique, multi-photon microscopy has make it possible to analyze deep portion of tissues in living animals such as axons of spinal code. Traumatic spinal cord injuries (SCIs) are usually caused by contusion damages. Therefore, observation of spinal cord tissue after the contusion injury is necessary for understanding cellular dynamics in response to traumatic SCI and development of the treatment for traumatic SCI. Our goal is elucidation of mechanism for degeneration of axons after contusion injuries by establishing SCI model and chronic observation of injured axons in the living animals. Firstly we generated and observed acute SCI model by contusion injury. By using a multi-photon microscope, axons in dorsal cord were visualized approximately 140 micron in depth from the surface. Immediately after injury, minimal morphological change of spinal cord was observed. At 3 days after injury, spinal cord was swelling and the axons seem to be fragmented. At 7 days after injury, increased degradation of axons could be observed, although the image was blurred due to accumulation of the connective tissue. In the present study, we successfully observed axon degeneration after the contusion SCI in a living animal in vivo. Our final goal is to understand molecular mechanisms and cellular dynamics in response to traumatic SCIs in acute and chronic stage.

8947-8, Session 2

Chromophore behavior in aging bruises

Richelle J. M. Hoveling, Ton G. van Leeuwen, Maurice C. Aalders, Academisch Medisch Ctr. (Netherlands)

The determination of the age of inflicted bruises is one of the important diagnostic factors in child abuse cases. This is currently based on subjective methods where the color of the bruise is compared to standardized color charts. We developed a 3D finite element model based on convection, diffusion and enzymatic conversion of the known chromophores in a bruise (hemoglobin and bilirubin) for the objective and accurate determination of the age bruises using hyperspectral imaging. [1] To refine and improve this model the chromophore behavior is further investigated by:

1 Addressing the influence of gravity on the diffusion of the different chromophores. For this we will inject hemoglobin and different forms of bilirubin in fresh tissue specimen and image their (direction of) diffusion using a hyperspectral camera (FTS, 400-720 nm).

2 Determining the influence of skin optical properties on the measured spectra by implementation of light propagation physics to predict the color appearance of bruises.

3 Studying the influence of varying layer thickness and chromophore concentrations using a mixture of scattering and absorbing media in tissue equivalent phantoms.

Results show the influence of gravity on the diffusion of the chromophores in different tissue structures. In the tissue equivalent phantoms a change in the color appearance of the chromophores is observed for increasing thickness of the surface layer. Based on these

results the model is further refined to improve the accuracy of the age determination of bruises. This will be illustrated using examples of typical bruises.

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8947-9, Session 2

Temporally and spectrally encoded confocal microscopy (T-SECM)

Jaehyun Hwang, Soocheol Kim, Jung Heo, SuHo Ryu, Chulmin Joo, Yonsei Univ. (Korea, Republic of)

Spectrally encoded confocal microscopy (SECM) is an imaging modality capable of high-resolution, three-dimensional imaging of biological tissues. SECM employs a diffraction grating to disperse a broadband light, so that each wavelength maps onto a position along one axis in a specimen. By measuring the reflected light via spectrally resolved detection, the information along the axis is captured, only requiring scanning in the other direction for image construction. Owing to its single-mode fiber based implementation, it has been constructed into a small endoscope, allowing for in vivo evaluation of cellular features. Clinical imaging at cellular scales, however, requires even more improvement in speed and implementation to reduce motion artifacts.

We developed a novel approach of SECM, or T-SECM, that can potentially enhance imaging speed. T-SECM illuminates a specimen with the lines of different wavelengths. The spatial coordinates along the wavelength-dispersion axis is measured by spectrally resolved detector, while those along the other axis is mapped into different modulation frequencies. Therefore, it does not involve beam scanning across the specimen, providing the potential for higher imaging speed. We implemented a T-SECM prototype based on a 50 kHz wavelength-swept laser and a single InGaAs photo-detector. The measured field of view was > 500 um x 500 um, and the spatial resolution was measured to be < 2um.

In the talk, we will describe the concept and implementation of T-SECM, along with its performance characteristics. We will also demonstrate its three-dimensional imaging capability by showing depth-resolved images of biological tissues

8947-10, Session 2

Imaging infrared spectroscopy for fixation-free liver tumor detection

James V. Coe, Heather C. Allen, Charles L. Hitchcock, The Ohio State Univ. (United States)

Infrared (IR) imaging spectroscopy of human liver tissue slices has been used to identify and characterize liver tumors. Liver tissue, containing a liver metastasis of breast origin (mucinous carcinoma) was surgically removed from a consenting patient and frozen without formalin fixation or dehydration procedures, so that lipids and water remain in the tissues. A set of IR biomarkers (ratios of various IR peaks) was determined for tumors in fixation-free liver tissues. K-means cluster analysis was used to tell tumor from nontumor. In this case, there was a large reduction in lipid content on going from nontumor to tumor tissue and a well resolved IR spectrum of nontumor liver lipid has been obtained and analyzed. These IR biomarkers may someday guide work on IR spectroscopic diagnostics on live patients in the operating room. This work also suggests utility for these methods beyond the identification of liver tumors, perhaps in the study of liver lipids.

8947-11, Session 2

High-speed stimulated Raman spectral imaging for digital staining of mouse cancer tissues

Yoichi Otsuka, Shuya Satoh, Masafumi Kyogaku, Hiroyuki Hashimoto, Canon Inc. (Japan); Kazuyoshi Itoh, Osaka Univ. (Japan); Yasuyuki Ozeki, Univ. of Tokyo (Japan)

We have been developing the high-speed stimulated Raman scattering (SRS) microscope, which enables us to observe biological specimens without chemical labeling. The existent system generates Raman images at the speed of 30 frames/sec with different Raman shift (ca. 300 cm-1 of bandwidth). We previously reported that the multivariate analysis techniques such as principal component analysis and independent component analysis are applicable for picking up tissue structures from SRS data of normal mouse and rat tissues (Ozeki et al., Nat. Photonics, 6, 845 (2012). Here, we report the multi-area observation of the tumor-grafted mouse tissue based on these approaches.

Tumor-grafted mouse (balb/cAcJ nu/nu) was prepared by injection of human pancreatic carcinoma cell line (SUIT-2) into the tail of pancreas. The tumor-grafted tissues were resected and fixed in formalin. Tissues were cryo-sectioned with a thickness of 100 um and observed. The data acquisition was continued for the 48 adjacent areas (500x500 pixels, 91 different Raman shift images for each area) and they were combined to analyze. For the efficient component analysis of large data set, an original protocol was developed.

The results indicated the different shapes and compositions between the tumor region and the normal region. The cells in the normal region indicated rounded shape and held small particles, which seem to be acinar cells that stored zymogen granules in cytoplasm. On the other hand, the cancer cells inside the cancer region indicated irregular alignment. These characteristic images suggested the possibility of the identification between the cancer and the normal tissues.

8947-12, Session 3

Monitoring cell-drug interaction by high-speed confocal Raman microscopy

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Live cell imaging was performed using a recently developed high-speed confocal Raman microscopy system. For various cell types, the tracking of cell-intrinsic Raman bands was conducted. The high-resolution Raman images with four minutes temporal resolution clearly delineated the intracellular distribution of biologically important molecules such as DNA, protein and lipid.

We used this system to investigate the effects of Bortezomib on multiple myeloma cells. Single RPMI8226 was monitored after the application of the drug. 30?30 Raman spectra were acquired over a 16μm?16μm area for each time point with 0.5s integration time. Three Raman images were acquired at 0hr, 1hr and 2hrs time points for the same cell after adding 50nM Bortezomib. From reconstructed protein Raman images, vesicle-like structures were observed at the cell boundaries. Furthermore, the observed morphological changes closely correspond to the accumulation of polyubiquitinated proteins reported from immunofluorescence staining experiments. Increased protein Raman signal corresponds to the well-known effects of proteasome inhibitors which lead to the abnormal intracellular accumulation of nonfunctional proteins and ultimately to apoptosis.

Based on this initial observation with a single cell, the experiment was

repeated using a larger group of cells (400 treated and 400 untreated). As the result, the treated group shows a significant separation based on the increased protein band while the aggregate cellular content remained constant.

The work reported here demonstrates that Raman imaging is a powerful tool for studying various biomedical problems in-vitro with little to no sample preparation and external perturbation to the biological system.

8947-13, Session 3

Beat frequency-multiplexed fluorescence lifetime measurements for high-speed confocal lifetime microscopy

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Fluorescence lifetime is an established parameter as a robust contrast agent for fluorescence imaging. We report a novel, low cost and highly tunable optical system for fluorescent lifetime measurements. Deemed lifetime imaging using frequency-multiplexed excitation (LIFE), a digitally-synthesized signal is used to drive an acousto-optic deflector, which is operated in a cat's eye configuration to produce a single laser excitation beam containing multiple beat frequencies in the MHz range. The fluorescence signal is then detected by a photomultiplier tube and is compared in the digital domain with a reference signal to recover the lifetime. By using multiple frequencies simultaneously to recover the sample phase response over a large bandwidth, accurate determination of the sample lifetime can be obtained at high speed without the need for sweeping the excitation frequency or using costly Pockels cells. As a proof-of-concept, we demonstrate phase measurements for selected lifetime standards that are probed simultaneously using ten distinct frequencies over a bandwidth of 48 MHz. We examine the lifetime of dye samples in various chemical environments, and compare these data to commercial time-domain fluorescence lifetime spectroscopy systems. This innovation has applications in high-speed fluorescence lifetime imaging microscopy, lifetime-based FRET assays, tumor margin detection, and DNA sequencing.

8947-14, Session 3

Joint end-member and spatial hypothesis testing for estimating the number of components in multispectral FLIM data

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Quantitative description of tissue composition based on its natural fluorescence is possible by unmixing Multi-spectral Fluorescence Lifetime Imaging Microscopy (m-FLIM) data. Hyper-spectral unmixing techniques can decompose m-FLIM data into fluorescence decay signatures (end-members) and their fractional contributions (abundances) in a sample. However, one major drawback for practical implementation is their dependence on prior knowledge of the number of components within the tissue sample. This information is often not available in practice, and its estimation is especially problematic when artifacts in the imaging devices and poor signal-to-noise conditions are present. Furthermore, it is particularly challenging when working with auto-fluorescence end-members due to their high similarity. Here, we propose a joint end-member and spatial hypothesis testing approach, which finds the number of components in the sample making a decision based on our previous work of blind estimation of end-members and their contributions in a sample. Starting from an initial assumption of two elements, the number of components is gradually increased until a joint test is violated. In the first step of the hypothesis testing, the linear independence of the estimated end-members at each stage is evaluated. Next, the spatial significance of the candidate end-members is analyzed based on the abundances. The proposed methodology along with our blind unmixing approach was validated using m-FLIM samples from in-vivo hamster oral mucosa, and the results were compared against tissue histopathology.

8947-15, Session 3

Effects of surrounding media viscosity and particle size on optical trapping of microspheres

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In this study, we investigated the effects of size and surrounding media viscosity on trapping of microspheres. A continuous wave ytterbium fiber laser with a 1064 nm wavelength was used to create an optical tweezers system to perform optical manipulation experiments. Briefly, the optical trap set up consisted of an inverted microscope containing a 100X oil immersion objective with a high numerical aperture through which the laser beam was passed and converged to form the optical trap. The laser beam was collimated, steered, and coupled to the microscope through the epifluorescence port. The laser power at the trap focal spot was determined by measuring the input power at the back aperture of the objective lens multiplied by the objective transmission factor at 1064 nm measured by a modified dual objective method. Polystyrene microspheres varying in diameter from 5 to 15 microns were suspended in liquid media in glass bottom petri dishes prior to trapping experiments. The microspheres were trapped at different trapping powers, and fluidic viscous drag forces where applied to the optically trapped microspheres by driving a computer controlled 2D motorized microscope stage at known velocities. The drag forces were calculated at the point that the trapped microspheres fell out of the trap, based on the Stokes equation for flow around spheres. The preliminary data show a linear relationship between trapping force and trap power within the range of the microsphere diameters and suspension media viscosity values used in this study. The work includes calculation of the dimensionless trap efficiency coefficient (Q) at 1064 nm wavelength and the corresponding effects of media viscosity and microsphere size on that value. The results of this study elucidate optical determination of the motility forces of biological structures using optical tweezers.

8947-16, Session 4

Identification of inflammation sites in arthritic joints using hyperspectral imaging

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Inflamatory arthritic diseases have prevalence between 2 and 3% and might lead to joint destruction and deformation resulting in a loss of function. Since hand and finger joints are often affected, the progress of the disease severely affects the patient's quality of life. Pathology involved in arthritis includes angiogenesis, hyper-vascularization, hyper-metabolism and relative hypoxia.

Hyperspectral imaging was employed to study vascular structures in and around affected and healthy finger joints. Two hyperspectral, push-broom cameras were used (VNIR-1600, SWIR-320i, Norsk Elektro Optikk AS, Norway). Optical spectra (400nm – 1700nm) of high spectral resolution were collected from 11 patients with visible symptoms of active inflamatory synovitis in at least one joint. The control group consisted of 10 healthy individuals. The concentration of dominant chromophores was calculated based on analytical solutions of light transport in tissue (diffusion theory). Image processing was used to analyze hyperspectral data and retrieve information like, e.g. blood concentration, edema formation, tissue oxygenation, and vascular structure. The presented data can be used to gain better understanding of how optical properties vary in inflamed joints. Knowledge of quantitative changes within affected joints and surrounding tissue will have positive impact for diagnosis of arthritic joints in the early stage. Moreover it will enable further development of fast and noninvasive diagnostics of arthritis. Results of these studies show that hyperspectral imaging can be a promising, new, and noncontact tool for characterization of the arthritic joints.

8947-17, Session 4

Toward in-vivo diagnosis of skin cancer using multimode imaging dermoscopy (SkinSpect): (I) clinical system development and validation

Nicholas B. MacKinnon, Fartash Vasefi, Spectral Molecular Imaging Inc. (United States); Daniel L. Farkas, Spectral Molecular Imaging Inc. (United States) and Univ. of Southern California (United States)

Purpose: To develop and evaluate the performance of a new clinical multimode dermoscope (SkinSpect™) for extracting skin chromophore concentrations in vivo.

Materials and methods: The SkinSpect imaging system combines fluorescence, polarization control and hyperspectral imaging technologies with 30 - 50 wavelength bands available between 480 nm and 900 nm. In our previous research prototype, the reflected photons were divided between parallel and cross polarized image paths and hyperspectral data sets were stored for further analysis. In the current clinical prototype, the reflected photons are no longer divided but are transmitted through a single optical path employing a liquid crystal variable retarder (LCVR) for polarization selection and a single image sensor.

Results: Optical design specifications of the clinical prototype are presented and compared with the previous prototype. Performance of the LCVR is characterized and analyzed. Spectral programming of the light source (OneLight®) is optimized to improve the accuracy of skin chromophore quantitation. Polarized attenuation spectra were computed from parallel and cross-polarized reflectance datacubes and then input into a wavelength-dependent linear model to extract the relative contributions of chromophores according to Beer-Lambert. The total hemoglobin concentration, oxygen saturation, concentrations of melanin and optical path-length were derived and compared for normal and pigmented nevus regions.

Conclusion: We have used SkinSpect's polarization-sensitive hyperspectral imaging system as a new, advanced, clinical dermoscope for in vivo skin analysis. The resulting data suggest that the system is useful for extracting and quantifying relative chromophore concentrations in skin tissues, especially highly pigmented lesions, in a topologically resolved manner.

8947-18, Session 4

Toward in-vivo diagnosis of skin cancer using multimode imaging dermoscopy (SkinSpect™): (II) molecular mapping of highly pigmented lesions

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Purpose: To propose and evaluate the performance of a quantitative imaging approach that combines two depth-sensitive imaging techniques, polarization and hyperspectral imaging, to produce a new multimode dermoscopy method that accurately maps melanin and hemoglobin oxygenation distribution in human skin.

Materials and methods: A rapid algorithm has been developed that uses two linearly polarized hyperspectral image sets to cancel out the response attributed to superficial melanin, and scattering. The method quantitatively maps the relative distribution of deep melanin, hemoglobin, and the spatially-resolved oxygen saturation in the skin.

Results: The algorithm was evaluated on skin with melanocytic nevus, vitiligo, and venous/arterial occlusion conditions. The results were compared with other ratiometric spectral imaging approaches. The analysis showed that this algorithm can quantitatively assess both hemoglobin content and regional oxygenation saturation independent of the melanin content of the skin. Access to the broad range of hyperspectral data in the visible and near-infrared range allows the algorithm to flexibly use different wavelength ranges for chromophore estimation while minimizing melanin-hemoglobin cross-talk.

Conclusion: This dermoscope and its quantitative skin analysis algorithm allows us to characterize highly pigmented lesions more accurately, and is to improve detection of potentially cancerous lesions.

8947-19, Session 4

Darkfield microscopy hyperspectral imager to detect single nanoparticles in breast cancer cells

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When combined with hyperspectral imaging, darkfield microscopy can be used to analyse nanoparticles in biological samples to determine the composition and the location of nanomaterials embedded in cells.

In this presentation, we demonstrate the capabilities of IMA™, Photon etc hyperspectral imager, in the analysis of nanomaterials in biological systems. As an example of biological specimens, we show spectrally resolved images of a sample of MDA-MB-231 human breast cancer cells tagged with 60 nm gold nanoparticles and exposed to darkfield illumination. With a 60? objective, an area of 150?110 ?m was imaged in a few minutes, covering the 400-650 nm spectral range, with a spectral resolution of 2 nm¬.

The high throughput and excellent optical imaging performances of Photon etc. hyperspectral filter allow the fast acquisition of spectrally resolved images of single nanoparticles. Since the camera captures the whole area in the field of view, it is possible to collect spectral and spatial information in real time, with the possibility of recording spectrally resolved videos to follow the dynamics of cells and luminescent nanoscale components. A single wavelength of the whole image is

filtered and focused on a CCD camera where a monochromatic image is formed. A spectrum within each pixel of the field of view is captured and spectral unmixing can be applied to classify the different types of nanoparticles in the sample.

When equipped with a darkfield condenser, IMA™, Photon etc. hyperspectral imager, can easily take part in routine analysis of nanoparticles in fixed or moving cells.

8947-20, Session 4

Multispectral imaging for diagnosis and treatment

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A new approach for generating high-speed multispectral images has been previously reported by our team. The central concept is that spectra can be acquired for each pixel in a confocal spatial scan by using a fast spectrometer based on optical fiber delay lines. The spectrometer uses a serial array of reflecting spectral elements, delay lines between these elements, and a single element detector. This approach merges fast spectroscopy with standard spatial scanning to create datacubes in real time. The datacubes can be analyzed to define regions of interest (ROIs) containing diseased tissue. These segmentations are based on ratios of fluorescent intensity in various spectral bands, and also occur in real time. Firmware and software have been developed for selectively scanning these ROIs with increased optical power. One can imagine three sequential scans that would detect disease, treat disease, and confirm the treatment. This achieves a tight coupling – both spatially and temporally – between detection and intervention. The approach would enable real time treatment with a spatial resolution of a few microns. An initial demonstration of the approach is presented using known test specimens. (Partially Funded by NIH SBIR Phase II Grant # 5R44CA124036-03)

8947-21, Session 4

Bartonella henselae invades of human erythrocytes in vitro

Gislaine Vieira-Damiani, Univ. Estadual de Campinas (Brazil); Marna Elise Ericson M.D., Univ. of Minnesota (United States); Vitor B. Pelegati, André A. de Thomaz, Hernandes F. Carvalho, Carlos Lenz Cesar, Marilene Neves, Tânia Benetti Soares, Paulo E. F. Velho, Univ. Estadual de Campinas (Brazil)

Bartonella ssp as the agent of cat scratch disease, bacillary angiomatosis, peliosis hepatis, endocarditis and bacteremic syndrome in humans. B. henselae infects cat and dogs and it provokes an intra-erythrocytic bacteremia in these animals.

The methods used in study of intraeritrocitic B. henselae can be by electron microscopy or by immunofluorescence. However, it is techniques that require processing and fixation of the material and thus bacterial killing. The ability to analyze in vivo, opens the door to understanding the interaction between B. henselae and erythrocyte enabling future studies may clarify the mechanisms of bacterial entry into host cells.

The goal of this study was to investigate through fluorescence microscope the presence of B. henselae in mature human erythrocytes without marker (immunofluorescence).

One sample of red blood cell (RBC) received an experimentally standard strain of B. henselae. Blood sample unstained from infected and uninfected was diluted with buffer PBS 1/10 was analyzed by fluorescence microscope. The sample fluorescence was excited with a 488nm Argon laser [Lasos, model LGN3001], from ZEISS LSM 780, with 40xN.A. 1.3 oil immersion objective (EC Plan Neofluar), with 512 ? 512 pixels spatial resolution, using a pixel dwell time of 12,6 µs, with total scanning time of order of 3s. TPEF (two photon excitation fluorescence) was collected in the backscattering mode and signal detected with Zeiss LSM 780 scan head photomultiplier tubes (PMT).We observed presence of B. henselae mature human erythrocytes without marker (immunofluorescence). This study demonstrates that B. henselae and invades mature human erythrocytes.

8947-22, Session 4

Holographic approach for monitoring of the deactivation of excited biomolecules

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The major direct method applied for detection of excited biomolecules is the recording of a fluorescence signal induced by their radiative deactivation. However for many of important molecules the radiationless channel of deactivation prevails making thus the fluorescence signal intensity very low. In such cases indirect methods may be used being based on registration of thermal variations in a medium induced by the radiationless release of energy. One of the most commonly used methods is the thermal lens technique. Although it allows one to monitor temporal characteristics of a process, it does not provide information on spatial distribution of thermal disturbances.

We suggest a novel approach based on the technique of holographic interferometry which allows one to obtain in a single shot a 2D image of the whole area under study. The recorded patterns provide data on spatial distribution of local variations of refractive index induced by temperature gradient.

In our experiments the technique was applied to monitor the process of photosensitized generation and following radiationless deactivation of singlet oxygen in water. The recorded holographic interferograms allowed us to reconstruct the temperature field in the area under study and thus to obtain information on the spatial distribution and dynamics of excited oxygen molecules. Being combined with fluorescence detection the holographic technique can provide a comprehensive data both on spatial distribution and temporal evolution of excited biomolecules in a medium.

8947-66, Session PMon

Diffusion optical spectroscopy of cancerous and normal prostate tissues in time-resolved and frequency domain

Kenneth J. Zhou, Stony Brook Univ. (United States); Lin Wang, Columbia Univ. Medical Ctr. (United States)

It is well-known that light transport can be well described using Maxwell's electromagnetic theory. In biological tissue, the scattering particle density is such that the interaction of scattered waves from neighboring particles cannot be ignored; therefore, multiple scattering occurs. The theoretical solution of multiple scattering is complicated. A suitable description is that the wavelike behavior of light is ignored and the transport of an individual photon is considered to be absorbed or scattered. This is known as the Radiative Transfer Equation (RTE) theory. Analytical solutions to the RTE that explicitly describes photon migration can be obtained by introducing some proper approximations. One of the most popular models used in the field of tissue optics is the Diffusion Approximation.

In this study, we report on the results of our initial study of optical properties of ex vivo normal and cancerous prostate tissues and how

tissue parameters affect the near infrared light transporting in the two types of tissues. The time-resolved transport of light is simulated as an impulse isotropic point source of energy within a homogeneous unbounded medium with different absorption and scattering properties of cancerous and normal prostate tissues. Light source is also modulated sinusoidally to yield a varied fluence rate in frequency domain at a distant observation point within the cancerous and normal prostate tissues. . Due to difference of the absorption and scattering coefficients between cancerous and normal tissues, the expansion of light pulse, intensity, phase are found to be different.

8947-67, Session PMon

Excitation and emission spectra criteria for distinguishing human Barrett's esophagus, normal and adenocarcinoma

Kenneth J. Zhou, Stony Brook Univ. (United States); Lin Wang, The Herbert Irving Comprehensive Cancer Ctr. (United States) and Kunmin Medical College (China)

Early detection of a premalignant Barrett's esophagus is critical for the success of cancer therapy. The aim of this ex vivo study was to evaluate the ability of fluorescence spectra to identify different types of Barrett's esophagus tissues. Twenty-four pieces of adenocarcinoma, five pieces dyplasia, and ten pieces of Barrett's esophagus tissue with pathologically confirmed Barrett's esophagus, as well as thirty pieces of normal tissues underwent excitation and emission spectra measurements with selective wavelength. The emission spectra of resected fresh tissue were obtained by 340 nm excitation to study the relative changes of collagen and NADH and the excitation spectra were acquired with 380 nm emission to compare the contribution from tryptophan and collagen. The spectra were analyzed by blind source separating method to predict which kind of tissues they were. Predictions were compared to the gold standard of histopathology. The results indicate that these key fluorophores within tissue, e.g. tryptophan, collagen, and NADH, and flavin, show differences of relative contents of fluorophores among different types of esophagus tissues, which reflects that the native excitation and emission spectroscopy measurements are effective for detecting changes of fluorophores composition in Barrett's esophagus tissues due to the developments of dysplasia and cancer. This ex vivo preliminary trial demonstrates that criteria obtained with excitation and emission spectra can distinguish neoplasia and carcinoma from normal Barrett's esophagus with good sensitivity and specificity.

8947-68, Session PMon

Fluorescence lifetime imaging of lipids during 3T3-L1 cell differentiation

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Obesity is becoming a big health problem in these days. Since increased body weight is due to increased number and size of the triglyceride-storing adipocytes, many researchers are working on differentiation conditions and processes of adipocytes. Adipocytes also work as regulators of whole-body energy homeostasis by secreting several proteins that regulate processes as diverse as haemostasis, blood pressure, immune function, angiogenesis and energy balance. 3T3-L1 cells are widely used cell line for studying adipogenesis because it can differentiate into an adipocyte-like phenotype under appropriate conditions. When various differentiation conditions of 3T3-L1 cells into adipocytes are studied, fluorescence imaging is normally used to monitor lipid droplets in them. However, there are also many lipids in cell membrane and it is hard to distinguish them from lipid droplets especially at the early stage of an adipocyte differentiation process. In this paper, we propose an effective fluorescence lifetime imaging technique which can easily discriminate between membrane lipids and lipid droplets. Nile red dyes are attached to lipids in 3T3-L1 cells. Fluorescence lifetime images were taken for 2 week during differentiation procedure of 3T3-L1 cells into adipocytes. We used 488 nm pulsed laser with 5MHz repetition rate and emission wavelength is 520 nm of Nile Red fluorescent dye. Results clearly show that the lifetime of Nile red in lipid droplets are smaller than those in cell membrane. Our results suggest that fluorescence lifetime imaging can be a very powerful tool to monitor lipid droplet formation in adipocytes from 3T3-L1 cells.

8947-69, Session PMon

Effect of blue/red LED light combination on growth and morphogenesis of saccharum officinarum plantlets in vitro

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The importance of light in the development of plants it is well established nowadays. It is well known that light intensity, wavelength(light quality), and photoperiod regulate growth, differentiation, morphogenesis of plant cells, and tissues cultures. For in vitro culture conventional fluorescent lamps, metal halide, high-pressure sodium, and also incandescent lamps are employed in order to obtain high photosynthetic-photon-flux(PPF). However, these conventional sources exhibit no parameters control and produce undesirable wavelengths for promoting growth. In order to overcome these drawbacks of conventional illuminants, light-emitting diodes(LEDs) have recently emerged as the ideal sources for in vitro culture of plantlets owing to their several advantages including controllable emission wavelength and light intensity, spatial power distribution, and polarization. The LED-based sources also possess valuable properties such as low power consumption, high electrical energy to light conversion efficiency, long-life, and low-cost and easy maintenance, and also may provide comparable PPF. Moreover, they incorporate environmental advantages because their production requires no emission of greenhouse gases (CO2), and provokes no mercury pollution. LED-based lighting systems have already successfully substituted conventional illuminants in several in vitro plantlet cultures including cotton, bananas, grapes, strawberries, potatoes, and maize, promoting better growth, as reported by many. Recently, investigations examining the effect of red, blue, and red-blue light mixture on the growth and morphogenesis of upland cotton, and rapeseed in vitro plantlets have been presented. However, little attention has been devoted to the investigation of monochromatic red and blue and bi-chromatic red-blue mixture on in vitro biofuel plantlets species. The effect of blue and red light mixture and white-light(RGB) from monochromatic light-emitting diodes on growth and morphogenesis of sugarcane(Saccharum officinarum) plantlets in vitro, was investigated. Light treatments with blue/red light intensity percentage ratio 70/30, 50/50, 40/60, 30/70, and also white-LED light were applied during a period of 20 days with a photoperiod of 16h per day. Results indicate that the blue/red light blend ratio of the illumination system plays a major role in fresh weight, length, and shoot multiplication of plantlets cultured in vitro. White-light via blended primary colors monochromatic LEDs illumination of the plantlets culture was also evaluated and compared with blue/red lighting

8947-70, Session PMon

Cytometric analysis of retinopathies in retinal trypsin digests

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Objective: To design an automated image cytometry tool for determination of various retinal vascular parameters including extraction of features that are relevant to postnatal retinal vascular development, and the development and progression of diabetic retinopathy.

Materials and methods: A program which measures six unique parameters from retinal wholemount trypsin digests was designed. From the wholemount images, the software segments the two different vascular cell types, endothelial cells (EC) and pericytes (PC), determines the number of each cell type, reports the total number of cells in each field, and gives the ratio of EC to PC (E/P ratio). The software utilizes the retinal vascular images to also measure vessel caliber and coverage, identify intersection point locations, and calculate the total number of acellular capillaries. Acellular capillaries in the retina arise from chronic exposure to hyperglycemia, have no cell nuclei and exhibit a very small width. We compared these parameters in images from wild type, transgenic, and diabetic mice. Retinal trypsin digests from bcl-2-deficient (bcl-2 -/-) mice, thrombospondin-1-deficient (TSP1-/-) mice, and diabetic Akita/+; TSP1-/- mice were used for these analysis and compared with wild type mice.

Results: Images from wild type and transgenic mice trypsin digests were compared. Significant differences were observed in all the measured parameters of retinal vasculature from mutant eyes compared with wild type of same age. The trypsin digest images showed that the retinas from diabetic mice had fewer vascular cells than the retinas from non-diabetic mice. Using the vascular images, the software also showed greater vessel coverage, vessel caliber, higher number of branch points and acellular capillaries in diabetic retinas. The comparison of retinal trypsin digest from bcl-2 -/- and TSP1-/- mice also showed significant differences in the analyzed parameters when compared with retinas from wild type mice of same age. Classification was performed on the extracted features using a majority vote between a linear classifier, k-nearest-neighbors classification, and a support vector machine. Classification accuracy of 88% was determined using the leave-one-out cross-validation technique.

Conclusions: We have developed an image cytometry program capable of quantifying retinal vascular health from images of the retinal trypsin digest, and classifying a retina as normal or injured. Automated image cytometry tools allow for feature extraction of retinal vasculature from diabetic and mutant mice for high throughput analysis more reliably compared to manual evaluation, a tedious task and prone to error. In addition, since the software is sensitive to minor changes in the vasculature and cellular distribution, it will allow for more accurate diagnosis of injury, and ultimately more effective treatment.

8947-71, Session PMon

Ptychography: use of quantitative phase information for high-contrast label free time-lapse imaging of living cells

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The uses of fluorescent dyes are well established for enhancing contrast of living cells. Although they have been designed to minimise damage to the cell, ultimately they can perturb the natural functionality. Techniques such as DIC and Zernike phase contrast are used to enhance contrast, however phase contrast produces undesired halo artefacts and both DIC and phase contrast have limited grey levels between the cell and the background, which limits image analysis software to perform cell segmentation.

Here we report a novel stain free, high contrast and quantitative method for imaging live cells. The technique reconstructs an image from overlapping diffraction patterns using a ptychographical algorithm (Maiden and Rodenburg, 2009; Maiden et al., 2010). The algorithm utilises both amplitude and phase data from the sample to report on quantitative changes related to the refractive index (RI) and thickness of the specimen.

We have previously demonstrated robustness of this technique to quantitatively analyse cells during mitosis (Marrison et al., 2013). In the present study ptychography was utilised to measure finer detail such as cell volume. Here we show a strong correlation between cell volumes as measured by confocal microscopy and the integrated phase shift using ptychography. We also report the power of this technique to image neurite elongation in neuronal cells; for detailed cell migration analysis; and to provide an accurate measure of cell proliferation and apoptosis.

The advantages of this label-free technology are especially significant for drug discovery, cancer research and primary/stem cell research since the cells can be studied in a non-toxic environment.

8947-72, Session PMon

Probing complex of influenza hemagglutinin with neutralizing antibody using terahertz spectroscopy technology

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Terahertz spectroscopy is sensitive to probe several aspects of biological molecules. We have reported the terahertz dielectric spectra was able to identify the type of the charges in the hydrogen-bonded antibodies' networks in our previous work. Recently we demonstrate a highly sensitive terahertz time-domain spectroscopy method to monitor binding between influenza hemagglutinin (HA) proteins and its primary target antibody F10-like Q1. The terahertz dielectric properties of HA was strongly affected by the presence of a specific antibody. Molecular arrangement or even concentration can also affect the signal. For instance, by increasing the concentration of the HA in solution we can deduce the hydration shell thickness from the concentration dependent non-linearity in our studies. With regard to molecular arrangement, terahertz spectra of HA is sensitive to the concentration variable neutralizing antibody F10-like Q1. We found that the corresponding absorption coefficient is significantly higher than the bulk HA protein after Q1 was added in and also presents the prominent distribution signature in terahertz dielectric spectra. The detectable minimum concentration of the HA protein was down to 0.0075mg/ml using the terahertz time-domain spectroscopy method. Furthermore, by increasing the concentration of the HA protein in antibody solution we can deduce the number of the effective recognize sites in the stalk region of HA which can be efficiently identified and targeted by the neutralizing antibody.

8947-73, Session PMon

Real time monitoring of superoxide dynamics in vivo through fluorescent proteins using a sensitive fiber probe

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Reactive oxygen species (ROS) are a group of highly reactive oxygen-derived molecules which can readily oxidize other molecules. They are currently appreciated as essential signaling molecules to regulate a wide variety of physiology. However, a wide range of distinct clinical disorders, such as cancer, aging, inflammation, etc., have been attributed to high levels of ROS. Most intracellular ROS are derived from superoxide anion, which is the primary oxygen free radical generated in mitochondria. Current method can only quantify superoxide in vitro and real time monitoring is not possible due to the lack of a reversible superoxide-specific indicator. A method capable of directly monitoring superoxide concentration in vivo in real time can significantly advance our understanding on its pathophysiology.

Recently, a circularly permuted yellow fluorescent protein (cpYFP) was developed as a reversible superoxide-specific indicator. We have engineered transgenic zebrafish (Danio Rerio), which has similar

genetic feature as mammal, to specifically express the cpYFP in liver cells. We use a double-clad fiber-optic probe to noninvasively investigate the superoxide dynamics in vivo in real time through the fluorescence intensity. The fiber probe not only enhances the signal collection efficiency but also confines the observation area to reduce noise background. Several superoxide-inducing or scavenging reagents are administrated onto the fish to study their efficacy. The distinct biochemical pathways of the reagents can be identified in the transient behaviors of the time courses. This technique has the potential to be used as a high throughput in vivo pharmaceutical screening platform.

8947-74, Session PMon

Fluorescent cyanine probe for DNA detection and cellular imaging

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Carbazolescaffold with fused aromatics have recently attracted intense interest because of its biological active property.[1]To date, a variety of carbazole derivatives from various natural or synthetic sources have been used as fluorescence probes and antitumor drugs. Carbazole-based cyaninefluorophoresare highly sensitive and efficientfluorescent light-up probes for DNA.The properties, such as water solubility, biocompatible, hypotoxicity, enable them to stain living tissues and cells.[2]Vinylpyridiniumcarbazolederivatives have been published as specificfluorescent labels for nuclear DNA and subcellular structures.[3]

In our study, we have designed and synthesized a new type of carbazole-based cyanine which has been used to detect DNA or stain living cell. It shows large stokes shift and the emission maximum is over 600nm. The remarkablefluorescence enhancement and significantinduced CD signal were observed when the molecule interacts with DNA, which indicated the high binding affinity and highlightedits potential as a fluorescence light-up DNA probe.The evaluation with two-photon excited fluorescence (TPEF) method revealed the largetwo-photon absorption (TPA) cross section which enables its applicationin TPEFimaging for living tissues and cells.This study indicates thatthis kind of carbazole-based biscyaninewould be good candidate for bio-imaging to investigate the molecular behavior in living tissues and cells by using linear and nonlinear microscopy techniques.

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8947-75, Session PMon

Fluorescence interference contrast microscopy based approach to study real time interaction of melittin with plasma membranes

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Melittin is an anti-bacterial and hemolytic peptide found in bee venom. Cell lysis behavior of peptides has been widely investigated, but the exact interaction mechanism of lytic peptides with lipid membranes and its constituents has not been understood completely. In this paper we study the melittin interaction with lipid plasma membranes in real time using non-invasive and non-contact fluorescence interference contrast (FLIC) microscopy. Particularly interaction of melittin with plasma membranes was studied in controlled molecular environment, where these plasma membrane were composed of saturated lipid, 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) and unsaturated lipid, 1,2-dioleoyl-sn-glycero-3-phosphocholine(DOPC) with and without cholesterol. We found that melittin starts to form nanometer size pores in the plasma membranes shortly after interacting with membranes. But the addition of cholesterol in the plasma membrane slows down the pore formation process. Our results show that inclusion of cholesterol to the plasma membranes makes them more resilient towards pore formation and the eventual lysis of membrane.

8947-76, Session PMon

Fluorescent Cyanine Probe for DNA Detection and Cellular Imaging

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Various fluorescence probes have been developed with the development of the microscopy techniques. Molecule probes are of the most commonly investigated types in the bioimaging. However, it is difficult to do long time observation due to the photobleaching. Functionalized carbon nanomaterial has emerged as a new research scope due to the advantages of the carbon materials. The detonation nanodiamond (ND) has gained much attention due to the advantages of mass production, high photostability and nontoxicity. Functionalized ND has been investigated in the drug delivery system, toxicity test and the photostability. However, the systematic investigation of functionalized ND in the living cell labeling which would provide an evidence of the function of nanoparticles and the corresponding behavior of living cell, has been hindered.

In this study, we have modified the surface of ND and demonstrated the staining property of the ND in living Hela cell labeling. We also examined the intracellular localization in endocytic cells using confocal fluorescence microscopy and transmission electron microscopy. We demonstrated that the carboxylate ND can enter the living cell though the endocytosis process. The low toxicity and high photostability have also been clearly demonstrated. This study provides good prospect for the application of functional ND in living cell labeling or drug delivery.

8947-77, Session PMon

Assessment of the anti-cancer drug chemoresistance by Raman microspectroscopy and atomic force microscopy (AFM)

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Breast cancer metastasis suppressor 1 (BRMS1) is a protein mainly distributed around cellular nucleus that can suppress the metastasis of cancer without affecting the growth of the original cancer. Nuclear factor-kappa B activity and AKT phosphorylation are associated with BRMS1 implicated in chemoresistance. Anti-cancer drug doxorubicin (DOX) mainly interacted with cellular nucleus of cells. In this study, Raman microspectroscopy was used to compare the biochemical component differences, and atomic force microscopy (AFM) was to quantitatively detect the biomechanical properties among various cell types (231, 231/B, 435, 435/B and A549) treated with DOX. It was found that Raman spectra of different cancer cell lines were very similar with the presence of bands corresponding to DNA/RNA, lipids, proteins, and carbohydrates. The spectra of all cell types at nucleus had richer biochemical information

comparing with other positions (cytoplasm and cellular membrane). The principal component analysis of Raman spectra of the wild type and BRMS expressing cancer cells with the increase of DOX exposure time can be easily distinguished. The multiplex chemokine and cytokine analysis also indicated the inflammatory responses of these cancer cells to DOX. The results from AFM illustrated that Young's modulus and adhesion force of breast cancer cells expressing BRMS1 decreased after anti-cancer drug DOX exposure. This work has successfully demonstrated the capability of both Raman microspectroscopy and AFM in detection of chemoresistance of anti-cancer drug at single cell level.

8947-78, Session PMon

Revisit laser scanning fluorescence microscopy performance under fluorescence-lifetime-limited regime

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The holy grail of laser scanning fluorescence microscopy (LSFM) is to achieve high-resolution and high-speed imaging in order to permit studies of fast dynamical processes and to benefit high-throughput cellular characterization. While substantial progress has been made in the family of diffraction-limit-breaking nanoscopy tools, the real-time imaging speed of LSFM is still far from the intrinsic temporal limit – fluorescence lifetime. Nevertheless, continuing advances in ultrahigh-speed laser scanning solutions, along with emergence of ultrafast and sensitive photodetectors, imply that lifetime-limited LSFM might not be far from reach. Understanding the LSFM performance in this high-speed regime requires consideration of the fluorescence transient dynamics. Yet, such concern is typically missing in the existing LSFM theoretical model and is thus rarely fully explored.

Motivated by this, we revisit the LSFM performance, particularly in the speed regime approaching to the lifetime limit, by extending the LSFM theoretical framework, which takes both diffraction limit and fluorescence temporal response into account. We show that, in aberration-free LSFM under ultrafast laser-scanning operation, the image resolution is governed not only by the diffraction limit, but also the fluorescence dynamics that is mapped into space. More notably, our model, which also considers noise-limited performance, suggests that there still exists an order-of-magnitude gap between the current LSFM speed and the lifetime limit. An imaging frame rate of >100kHz could be viable with the emerging laser-scanning techniques using ultrafast wavelength-swept sources, or optical time-stretch. We anticipate the present framework could provide further insight in developing new modality of ultrafast LSFM.

8947-79, Session PMon

In situ monitoring of brain tissue reaction of chronically implanted electrodes with an optical coherence tomography fiber system

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Neural microelectrodes are well established tools for delivering therapeutic electrical pulses, and recording neural electrophysiological signals. However, long term implanted neural probes often become functionally impaired by tissue encapsulation. At present, analyzing this immune reaction is only feasible with post-mortem histology; currently no means for specific in vivo monitoring exist and most applicable imaging modalities provide no sufficient resolution for a cellular measurement in deep brain regions. Optical coherence tomography (OCT) is a well developed imaging modality, providing cellular resolution and up to 1.2 mm imaging depth in brain tissue. Further more, a fiber based spectral domain OCT was shown to be capable of a minimally invasive brain intervention.

In the present study, we propose to use a fiber based spectral domain OCT to monitor the tissue immune reaction progression and scar encapsulation of microprobes in a rat animal model. We developed a integrated OCT fiber catheter consisting an implantable ferrule based fiber cannula and a fiber patch cable. The fiber cannula was 18.5 mm long, including a 10.5 mm ceramic ferrule and a 8.0 mm long, ø 125 µm single mode fiber. A mating sleeve was used to fix and connect the fiber cannula to the OCT fiber cable. Light attenuation between the OCT fiber cable and the fiber cannula through the mating sleeve was measured and minimized. The fiber cannula was implanted in rat brain together with a microelectrode in "sight" used as a foreign body to induce the brain tissue immune reaction. Preliminary data were obtained and analyzed.

8947-80, Session PMon

Image and depth map quality metrics for phase contrast imaging

William A. Smith, Ka-Po Lam, James B. Richardson, Keele Univ. (United Kingdom)

High-resolution phase contrast microscopy facilitates quantitative, non-invasive and (thus) long term cell imaging and biometric studies. A key challenge with such studies is the capability of acquiring and identifying the best in-focus image which could accurately capture and reliably characterise cell behaviour with common cell events/activities including mitosis, apoptosis and translocation. The most common practice to date in assessing the quality of the resulting image is by visual inspection, which is both subjective and qualitative. Specifically, there is no 'gold standard' metric to assess the quality of the so-called in-focus or more precisely the reconstructed all-in-focus (AIF) image obtained from autofocusing procedures.

This study presents the analysis and evaluation of a number of the most commonly used image quality measures (IQMs) with the principal goal to establish an appropriate IQM for assessing the quality of phase contrast images. This is achieved by computing the IQMs for each AIF image generated from the z-stack. Surface/depth maps are then estimated by two different autofocussing algorithms are then evaluated with a surface smoothness metric (SSM).

The investigation presented offers the viability of the quantitative assessment of AIF images based on a biologically meaningful and computationally tractable method of IQM. More importantly, the results obtained will in turn facilitate the closely allied development of an objective measure of the quality of autofocusing procedures in terms of the estimated focus/surface depth map and the corresponding AIF image.

8947-81, Session PMon

In vivo quantitation of circulating tumor cells based on real-time confocal microscopy

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The number of circulating tumor cells (CTCs) in blood of cancer patients is a potentially useful indicator for precise monitoring of cancer metastasis, evaluation of cancer treatment and even early detection of recurrent cancer. Currently, the major strategy for CTC quantitation is using microfluidic chip isolating CTCs from drawn blood sample. However, this ex vivo quantitation of rare CTCs, one cell per 109 hematologic cells, from the limited volume of blood sample is fundamentally challenging and suffers extremely low sensitivity. Direct in vivo quantitation of CTCs in blood circulation, potentially from whole

body blood, is an attractive approach to increase sensitivity. In this study, we implemented custom-design video-rate confocal microscopy system based on fast-rotating polygonal mirror. The system could acquire images of 512x512 pixels at 30 frames/sec, which allowed us direct imaging of fast-flowing individual cells in great saphenous vein (GSV) of mouse model in vivo. After intravenous injection of CT26 colorectal cancer cells and RBC labeled with CFSE (green) and DiD (NIR) respectively, we clearly imaged fast circulating CT26 and RBC at GSV. The number of flowing CT26 rapidly decreased below 10% of initially detected number at 3 minutes after the injection, while the number of flowing RBC remained at initially detected number over 60 minutes. From the pre-determined total number of injected RBCs, we could calculate a relative calibration factor and estimated circulating CTC in whole body blood in vivo. Furthermore, we also repeatedly monitored CTCs disseminated from implanted tumor at footpad over 3 weeks.

8947-82, Session PMon

Image processing with the radial Hilbert transform of photo-thermal imaging for carious detection

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Knowledge of heat transfer in biological bodies has many diagnostic and therapeutic applications involving either raising or lowering of temperature, and often requires precise monitoring of the spatial distribution of thermal histories that are produced during a treatment protocol.

The present paper therefore aims to develop a mathematical algorithm using Hilbert transform for edge detection of photo–thermal imaging due to interactions between laser rays and biological tissues.

Photothermal imaging has the ability to penetrate and yield information about an opaque medium well beyond the range of conventional optical imaging. Owing to this ability, Q- switching Nd:YAG laser at wavelength 1064 nm has been extensively used in turbid media such as human teeth to study the sub-surface deposition of laser radiation. In this work, the optical and thermal properties of tissue are observed using frequency-domain infrared photothermal imaging and enable us to detect the carious position. The high absorption coefficient of the carious rather than normal region rise its temperature generating IR thermal radiation captured by high resolution thermal camera. Changing the pulse repetition frequency of the laser pulses affects the penetration depth of the laser, which can provide three-dimensional (3D) images in arbitrary planes and allow imaging deep within a solid tissue.

The Hilbert transform is useful for image processing because it can select which edges of an input image are enhanced and to what degree the edge enhancement occurs.

We introduce a radially symmetric Hilbert transform that permits two-dimensional edge enhancement to trace the carious position in the 3D photothermal images for carious removal.

8947-83, Session PMon

Atherosclerosis staging: imaging using FLIM technique

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Atherosclerosis is a progressive disease that begins in childhood and manifests clinically in middle to late adulthood. Atherosclerotic plaque formation occurs due the accumulation of lipids, and is the main cause of coronary artery disease [1]. It was observed protoporphyrin IX accumulation in atherosclerotic plaque[2]. In this work it was used Fluorescence lifetime imaging (FLIM) to analyze atherosclerotic plaque biochemical composition. For this purpose an animal experimentation was done with New Zealand rabbits divided into two groups: a control group of 4 rabbits that received a regular diet for 0, 20, 40 and 60 days; and an experimental group of 9 rabbits, divided in 3 subgroups, that were fed with 1% cholesterol diet (Sigma-Aldrich) for 20, 40 and 60 days respectively. The rabbit's aortas were analyzed by FLIM exciting samples at 440 nm. The results shown an increase in the lifetime imaging of rabbits fed with cholesterol. It was observed that is possible to detect the metabolic changes associated with atherosclerosis at an early stage using FLIM technique exciting the tissue around 440 nm and observing autofluorescence time duration. Lifetimes longer than 1.75 ns suggest the presence of porphyrins in the tissue and consequently, inflammation and the presence of macrophages. Acknowledgements: FAPESP 2010/16544-1, INCT Fluidos Complexos.

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8947-85, Session PMon

New blood markers for staging and prognostics of atherosclerosis

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Atherosclerosis is a cardiovascular disease with a multifactorial origin. The analysis of blood biomarkers helps to understand the formation of the plaque and its instability. The present study aims to evaluate the performance of three atherosclerosis fluorescent biomarkers: europium-chlortetracycline complex (EuCTc) used to mark the low density lipoprotein [1], the Evans Blue (EB) recently used to localize oxidized low-density lipoprotein in human coronary artery [2] and Thioflavin T (ThT) considered a new method for assessing cardiovascular risk [3]. For staging the atherosclerosis progress, a total of 12 New Zealand rabbits were divided into three groups. Each group contained three animals in an experimental group (EG), and one animal for control group. The animals in the EG received a diet with 1% of cholesterol. Blood samples were collected with 0, 20, 40 and 60 days. The results from spectroscopy analysis showed that the emission intensity of EuCTc marker in the presence of plasma is proportional to the plaque's formation. The EB emission intensity remained constant. The ThT showed good results but it was required a large number of analyzes of the same sample, probably due to the short Stokes shift. The studied biomarkers may not yet be specific in the identification of unstable plaques, but can provide additional information on the patients risk for plaque formation. [1] L. D. S. Teixeira, Analytical Biochemistry, 400 (1):19-24 (2010). [2] Uchida Y, PLoS ONE 8(2), (2013). [3]M. D. W. Griffin, Clinical Biochemistry 43(3), 278-286 (2010).

8947-86, Session PMon

Efficacy of photodynamic therapy against larvae of Aedes aegypti: confocal microscopy and fluorescence-lifetime imaging

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Today, Aedes aegypti's population control is undoubtedly the most effective strategy available for disease prevention and containment of outbreaks. Among numerous alternatives to combat the vector,

photosensitizers have been used as larvae of Aedes aegypti inactivators. These drugs are able to interact with light to generate reactive oxygen species, e. g. singlet oxygen. In this work we report the phototoxic effects of photosensitizers, which may represent promising tools for future control of the dengue mosquito. The effectiveness of Photodynamic Therapy (PDT) against different instar of Aedes aegypti was evaluated using a hematoporphyrin-derivative, Photogem®, as photosensitizer. The larvae at 2th instar were exposed to different concentrations of the drug: 20 ?g/ml, 40 ?g/ml and 80 ?g/ml. After 30 minutes of exposure to Photogem®, it was observed the presence of drug in the gut of the larvae by confocal fluorescence images. Spectral confocal microscopy and fluorescence life time imaging (FLIM) were used to evaluate the interaction of the photosensitizer with the digestive tract of the larvae. FLIM images showed greater drug concentration in the intestinal wall of the samples, which produces a strong decrease of the Photogem® fluorescence decay time.Three different sources for the irradiation of samples were used: LEDs (630 nm), natural light and a fluorescent lamp (40W and intensity of 6mW). The results post-PDT showed a 93.33% mortality 48 hours after PDT in 4th instar larvae using a LED as light source, and 100% mortality 24 hours after irradiation with both natural light and fluorescent lamp. These results indicate a potent photodynamic effect against the larvae of Aedes aegypti.

8947-87, Session PMon

Quantitative microscopic Mueller matrix polarimetric imaging of red blood cells

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Optical properties such as refractive index of the red blood cells (RBCs) have been shown to change during progression of diseases such as malaria, cancer, and diabetes. Conventional optical techniques allow bulk measurements only on average behaviour of a cell population, losing the information on cellular heterogeneity. In order to identify more sensitive label-free optical markers for determining physiological state of the RBCs during ageing and disease progression, we carried out single cell polarimetric imaging of RBCs using Mueller matrix (MM) approach. Measurement of MM provides important polarization properties such as diattenuation, birefringence and depolarization. Each of these constituent polarization properties, if properly extracted and quantified, can potentially serve as useful biophysical and physiological metric. Here, we report development of a Mueller matrix based polarimetric microscopic imaging setup and its use for measurement of optical properties of RBCs. The system utilized liquid crystal variable retarders with polarizers and quarter-waveplates to generate and analyze the polarization states of the RBCs. The system was built on an inverted microscope platform and integrated with a CCD camera for mapping the polarization properties of the RBCs in the field of view. Use of Mueller matrix based polarimetric microscopic imaging for evaluation of physiological state of RBC will be presented.

8947-88, Session PMon

Toward noninvasive diagnosis of Duchenne muscular dystrophy by hyperspectral imaging: preclinical analysis of ex-vivo tissue specimens

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Purpose: To determine the performance of hyperspectral imaging for detection of ex-vivo mouse muscle tissue affected by Duchenne Muscular Dystrophy (DMD).

Methods: An angular domain hyperspectral imaging system was built and used to test fresh murine tissue samples with a thickness of 2 mm. The hyperspectral system consisted of a broadband lamp, an angular filter array (AFA), and an imaging spectrometer. The AFA consisted of a series of parallel micro-channels micro-machined in silicon and was placed between the sample and the imaging spectrometer. The AFA preferentially selected for ballistic and quasi-ballistic (snake) photons, which retained their original trajectories and rejected scattered photons to a large extent. The resultant image cubes were corrected from instrument responses as well as spatial artifacts due to AFA geometry.

Results: As a proof of concept, spectral data cubes (650 - 850 nm) were acquired from 2-mm thick murine tissue samples taken from 2 wild-type,control animals, three animals with mild DMD (mdx mice), and 2 animals with severe DMD (mdx:utrn-/- mice). The transillumination attenuation spectra were input into principal component analysis and discriminant analysis. The resulting maps discriminated between healthy muscle tissue and the DMD-affected tissue.

Conclusion: We have shown that hyperspectral trans-illumination imaging with multi-variant analysis can result in a distinction between healthy and DMD-affected muscle tissue. We propose that the training spectral vectors (principal component analysis) can be used to construct a set of classifiers to enable disease detection in samples and can be extended to a spectroscopic system for noninvasive DMD diagnosis.

8947-23, Session 5

Dental pulp stem cells (DPSCs) differentiation study by confocal Raman microscopy

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Regenerative medicine brings a huge number of applications for mesenchymal stem cells such as Dental Pulp Stem Cells (DPSCs). Their uses are conditioned by their differentiation status. New methods to determine the differentiation state of cells are of great value. Confocal Raman microscopy, a non-invasive, label free technic would be an ideal method to study osteogenic differentiation of DPSCs in situ. Cells were observed in PBS at room temperatude. Integrated Raman intensities in the 2800-3000 cm-1 region (C-H stretching) and 960 cm-1 peak (v1PO4 3-) were studied. Images revealed high phosphate band intensities around cells and high C-H signal in cells after culture in osteogenic media. Average spectra of the two different regions show clearly the difference in the extracellular and cellular compartment. In the extracellular spectra the phosphate peaks ?1PO43- (first vibrational mode) at 960cm-1 and ?2PO43 at 430cm–1 and ?4 PO43 at 585cm-1 are obviously present. In the cellular spectra the amide bands are higher than in the extracellular spectra Confocal Raman microscopy enables the detection of phosphate accumulation in the extracellular matrix and can be used to follow DPSC in bone cells.

8947-24, Session 5

The use of upconverting phosphors in point-of-care (POC) testing *(Invited Paper)*

Hans J. Tanke, Leiden Univ. Medisch Ctr. (Netherlands); Michel Zuiderwijk, Karien C. Wiesmeijer, Robert N. Breedveld, Leiden University Medical Center (Netherlands); William R. Abrams, New

York University College of Dentistry (United States); Claudia J. De Dood, Elisa M. Tjon Kon Fat, Paul Corstjens, Leiden Univ. Medisch Ctr. (Netherlands)

We have developed ultrasensitive Point-of-Care (POC) systems using upconverting (nano)phosphors (UCP) as reporter molecules. UCPs luminesce in the visible range of the spectrum upon excitation with infrared (980nm) light, are photostable and not hampered by quenching. As upconversion does not occur in nature, autofluorescence of assay components and of the biological material is circumvented, and very high signal-to-noise levels are obtained. We have applied these UCPs in lab-on-a-chip prototypes, that are handheld, low cost and easy to operate in remote settings. The detection platform is a lateral flow assay, driven by a microfluidics circuitry. For the simultaneous detection of different antibodies a specific lateral flow format was developed, referred to as consecutive flow (CF). CF is based on three successive flow steps which allow enrichment of the targets at spatially separated test zones. The CF format was successfully converted into a microfluidic device. Lateral flow strips are scanned (e.g. excited with infrared light) with a small-size detector (cellular phone format) to semi-quantify the results.

This technology has been applied to detect infectious disease (HIV, TB, HPV, schistosomiasis, lepra) in developmental countries. Recently, we also introduced this technique to monitor serum levels of immunotherapeutic drugs (adalimumab, infliximab) in patients with rheumatoid arthritis or Crohn's disease.

8947-25, Session 5

Fluorescent lifetime imaging of surgical specimens using two-photon microscopy at MHz rates

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Two photon fluorescence lifetime imaging (TP-FLIM) is a modality that enables high resolution, depth-sectioned intrinsic contrast imaging of the microenvironment of cells and tissue. In TP-FLIM, two photon excitation generates an excited state in a fluorophore which decays with a characteristic time constant that depends on the specific fluorophore, pH, temperature, local proteins and other characteristics. TP-FLIM therefore provides extensive information about the microenvironment of individual cells or entire tissues that may reflect metabolic changes associated with malignancy. However, because the TP-FLIM signal scales with the square of the excitation light intensity, TP-FLIM can only be performed under high numerical aperture, high magnification imaging. Combined with the extremely low pixel rates of existing TP-FLIM systems based on time correlated single photon counting (TCSPC), TP-FLIM has so far been limited to narrow field, low pixel number imaging. Consequently, generalizing the technique to the large field of view required for pathology imaging applications is challenging.

To address these limitations, we present a new method of performing TP-FLIM using high speed sampling of fluorescent excitation that enables sustained >1 MHz pixel rates, submicron resolution TP-FLIM. Using a custom microscope optimized for wide field of view (>5 mm^2) and rapid image mosaicing, we demonstrate gigapixel resolution lifetime imaging of excised human breast and thyroid cancer tissue. Images are compared with corresponding histology. TP-FLIM may enable real time assessment of surgical margins in cancer resection.

8947-26, Session 5

Lifetime modulated lanthanide tags

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Two types of Lifetime modulated lanthanide tags based upon proximity control based on acceptor concentration of non-radiative energy transfer have been developed. The first was used varying ratios of the trivalent europium complex Eu(TTFA)3 donor and Coumarin acceptor encapsulated into porous polystyrene beads. When in close proximity, a proportion of excited Eu(III)+ complexes transfer their energy to acceptor dyes, which leads to shortening of the luminescence lifetime of Eu(III) complex at the red emission band around 612 nm. Varying their concentrations stepwise changed the average donor-to-acceptor distance, making it possible to fine-tune the lifetimes of the microspheres in the range of 359 to-188 ?S.

The second approach is based on the change of luminescence life-time of lanthanide upconverting nanocrystals (tau-Dots), such as NaYF4:Yb,Tm. Lifetimes change is based upon varying the ratio of co-doping sensitizer Yb(III) ions and blue-emitting Tm(III) ions at stepwise varied concentrations into the NaYF4 nanocrystals. Energy transfer from the sensitizer to the emitter ion at a varying sensitizer-emitter distance also provides lifetime tunability. The lifetime of 40 nm NaYF4:Yb,Tm nanocrystals at blue emission band was tuned from 48 ?S (4 mol% Tm) to 668 ?S (0.2 mol% Tm). This yielded 10 nanocrystal populations having distinct lifetimes from 25.6 ?S to 662.4 ?S. Mixtures of multiple lifetime nanocrystals have ben embedded into Eu(TTFA)3 containing beads, which themselves can have multiple lifetimes. Individual nanocrystals and beads containing multiple nanocrystals can be used as tags which are applicable to multi-channel bioimaging, diagnosis, etc

8947-27, Session 5

High-throughput measurement of the long excited-state lifetime of quantum dots in flow cytometry

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The long excited-state lifetime of quantum dots (QDs) is not often utilized in high-throughput bioassays, despite of the potential for the lifetime to be an optimum parameter for multiplexing with spectrally overlapping excitable species that have short fluorescence lifetimes. The limitation of currently available instruments that can rapidly resolve complex decay kinetics of QDs contributes to this dearth. Therefore work in our laboratory is focused on developing unique and reliable frequency-domain flow cytometry (FDFC) systems as well as QD applications where fluorescence dynamics are exploited. In this paper we demonstrate both by simulation and experimental validation, the viability of rapidly capturing the fluorescence lifetime of QDs from single QD-labeled cells and microspheres by employing a home-built FDFC system. With FDFC theory we simulated measurements of long-lived QD decays and evaluated the potential to discriminate multi-exponential decay profiles of QDs from typical cellular autofluorescence lifetimes. Our FDFC simulation work included calculations of fluorescence phase-shifts at multiple modulation frequencies extracted from square wave modulation signals (i.e. similar to heterodyning frequency-domain spectroscopy). Experimental work to support the result from our simulations involved acquiring measurements from real samples and processing them for multi-frequency phase shifts. Additionally the average excited-state lifetimes of QDs (streptavidin conjugated CdSe/ZnS and oleic acid coated CdSxSe1-x/ZnS) measured were found to be greater than 15 ns. The average lifetime results were consistent with published literature

values as well as verified with independent time domain measurements. This work opens the possibility of developing powerful bioassays using FDFC based on the long fluorescence lifetime of QDs.

8947-28, Session 5

Android phone controlled handheld imaging system for biosensing applications

Khalid M. Arif, Olaf Diegel, Massey Univ. (New Zealand)

We present a handheld imaging device that interfaces with android phone for detection of cells and biomolecules. This device features integrated optics and electronics for imaging using LED white light and red diode laser. Optics is laid out in similar fashion as in optical pickup of compact disk drives, however the components are packaged in a custom designed and 3D printed casing. Onboard electronics and control unit consists of an arduino ADK board which provides all necessary communication as well as control functions. A high resolution CMOS image sensor interfaced with the board acquires images and transfers to the phone for processing. An application program running on the phone initiates data acquisition, processing and control of the device like any other touch-based android application.

Cell and biomolecule samples, prepared on bar-coded strips, are inserted into the device to activate it and initiate communication with the phone. A live image of the strip is shown when the user opens the application. The bar-code is also read to load relevant recipe and the system also populates the user interface with appropriate icons. Upon analysis, the user can readily share the results through email or other data sharing functions integrated into the application. The main goal of this system is to completely isolate the user from the underlying complexity of regular imaging and data analysis systems and/or experimental setups that usually require trained personnel.

We demonstrate application of the imaging system for cells, beads and gratings. Our findings suggest that this device can be used for medley of optical biosensing techniques.

8947-29, Session 6

Optical clearing based 3D visualization of cellular network in whole cortex of intact lymph node

Eunjoo Song, Howon Seo, Kibaek Choe, Yoonha Hwang, Jinhyo Ahn, Pilhan Kim, KAIST (Korea, Republic of)

Lymph node (LN) is an important immune organ where foreign pathogens are recognized and adaptive immune responses are initiated. To achieve prompt and efficient immune function, complex 3D cellular network composed of many subtypes of immune cells such as T, B cells and dendritic cells in conjunction with specialized lymphatic/vascular network are established in LN. Unfortunately, conventional histological analysis has critical limitations in 3D cellular network analysis due to structural disruption by chemical fixation and substantial tissue loss in slicing. Imaging techniques capable of sectioning such as laser-scanning confocal and two-photon microscopy have been utilized to analyze 3D structure of intact LN. However, light scattering within biological samples limits the imaging depth to only superficial portion of LN cortex. Optical clearing technique based on chemical agents have shown enhancement of imaging depth in various biological tissues, but their effects on LN are remained to be investigated.

Herein, we applied optical clearing technique and custom-built high-power laser-scanning confocal microscope to visualize cellular network in whole cortex of intact LN. To identify immune cellular network inside LN, we adoptively transferred many subtypes of immune cells purified from donor mice expressing various fluorescent proteins (CFP, GFP, YFP, DsRed) to wildtype recipient mouse. Also, we injected anti-CD31- or anti-LYVE1-antibody conjugated with NIR fluorophore to label vascular or lymphatic network. From the optically cleared LN, we successfully achieved 3D volumetric visualization of whole cortex of LN, which revealed major cellular structures such as T-cell zone, B-cell follicle, HEV, lymphatic sinus and germinal center.

8947-30, Session 6

Diagnosis of myocardial infarction based on lectin-induced erythrocyte agglutination: a feasibility study

Jozsef Bocsi, Kathleen Nieschke, Anja Mittag, Univ. of Leipzig (Germany); Thomas Reichert, GEMAK (Germany); Wiebke Laffers, Univ. of Bonn (Germany); Arkadiusz Pierzchalski, Joachim Piltz, Univ. Leipzig (Germany); Hans-Jürgen Esche, amtec Analysenmesstechnik GmbH (Germany); Günther Wolf, GEMAK (Germany); Ingo Dähnert, Attila Tarnok, Univ. Leipzig (Germany)

Myocardial infarction (MI) is an acute life-threatening disease with a high incidence worldwide. Aim of this study was to test lectin-carbohydrate binding-induced red blood cell (RBC) agglutination as an innovative tool for fast and precise diagnosis of MI.

Five lectins (Ricinus communis agglutinin (RCA), Phaseolus vulgaris erythroagglutinin (PHA), Datura stramonium agglutinin, Artocarpus agglutinin, Triticum agglutinin) were tested for agglutination characteristics in patients with MI or angina pectoris without MI (AP) and healthy volunteers (HV) as control. RBC agglutination was analyzed by light absorbance of a stirred RBC suspension for 15 min after lectin addition. Mean cell count in aggregates was estimated from light absorbance by a mathematical model.

Each lectin induced RBC agglutination. RCA led to the strongest RBC agglutination (~150 RBCs/aggregate), while the others induced substantially slower agglutination and lead to smaller aggregate sizes (5-20 RBCs/aggregate). Lectin-induced RBC agglutination of patients with MI or AP was generally higher than for HV. However, only PHA-induced agglutination clearly distinguished MI from HV. AP agglutination was intermediate (MI>AP>HV)

It is possible to differentiate between patients with MI and HP based on PHA-induced RBC-agglutination. We hypothesize that pathological changes induce modification of the carbohydrate composition on the RBC membrane and thus modify RBC agglutination. This novel assay could serve as a rapid, cost effective valuable new tool for diagnosis of MI.

Acknowledgement: This work was supported by Sächsische Aufbaubank (SAB No: 11624/1845) and BMBF Project MaDaKos (BMBF No.: 990101-088.12/2010-11/2013).

8947-31, Session 6

Flow cytometric assay for analysis of cytotoxic effects of potential drugs on human peripheral blood leukocytes

Kathleen Nieschke, Univ. Leipzig (Germany); Anja Mittag, Prima BioMed GmbH (Germany); Karolina Golab, The Univ. of Chicago (United States); Arkadiusz Pierzchalski, Univ. Leipzig (Germany); Wojciech Kamysz, Medical Univ. of Gdansk (Poland); Jozsef Bocsi, Attila Tarnok, Univ. Leipzig (Germany)

Toxicity test of new drugs belongs to the first steps in the drug screening, using different cultured cell lines. However, using primary human cells is more representative to the human organism. We developed a very gentle toxicity assay for isolation and incubation of human peripheral blood leukocytes (PBL) and tested it using different bioactive oligopeptides (OP).

Effects of different PBL isolation methods (red blood cell lysis; Histopaque isolation a.o.), different incubation tubes (e.g. FACS tubes), anticoagulants and blood sources on PBL viability were tested using propidium iodide-exclusion as viability measure (incubation: 60 min, 36°C) and flow cytometry. Toxicity concentration and time-depended effects (10-60 min, 36 °C, 0-100 µg /ml of OP) on human PBL were analyzed.

Erythrocyte lysis by hypotonic shock (dH2O) was the fastest PBL isolation method with highest viability (>85%) as compared to NH4Cl-Lysis (49%). Density gradient centrifugation affected neutrophil loss. Heparin anticoagulation yielded higher viability than EDTA. Conical 1.5 mL and 2 mL micro-reaction tubes (both polypropylene (PP)) yielded highest viability (99% and 97%), compared to three types of 5.0 mL round-bottom tubes PP (white-60%), PP (blue-62%), Polystyrene (PS-64%). Viability of PBL did not differ from venous as well as capillary blood. Using our assay OP-specific toxicity, time-course, dose-dependence and aggregate formation could be clearly differentiated and quantified.

A gentle reproducible preparation and analytical toxicity-assay for human PBL was developed and evaluated. This novel assay enables for rapid and cost effective multiparametric toxicological screening and pharmacological testing on primary human PBL and can be adapted to high-throughput-screening.

8947-32, Session 6

A high performance biometric signal and image processing method to reveal blood perfusion towards 3D oxygen saturation mapping

Ryan Imms, Sijung Hu, Vicente Azorin-Peris, Loughborough Univ. (United Kingdom); Michaël Trico, Polytech Paris-Sud (France); Ron Summers, Loughborough Univ. (United Kingdom)

Non-contact imaging photoplethysmography (iPPG) is a recent development in the field of physiological data acquisition, currently undergoing a large amount of research to characterize and define the range of its capabilities. Contact-based PPG techniques have been used in clinical scenarios for a number of years to obtain direct information about the degree of oxygen saturation for patients; with the advent of imaging techniques there is strong potential to enable access to additional information such as multi-dimensional blood perfusion and saturation mapping.

The further development of effective opto-physiological monitoring techniques is dependent upon novel modelling techniques coupled with improved sensor design and signal processing methodologies. The Loughborough University biometric signal and imaging processing platform (bSIPP), provides a comprehensive set of features for extraction and analysis of recorded iPPG data, enabling direct comparison with other biomedical diagnostic tools such as ECG and EEG.

Additionally, utilising information about the nature of tissue structure has enabled the generation of an electronic model describing the behaviour of light during its travel through the body. This enables the estimation of the relative oxygen saturation and blood perfusion in different layers of the tissue to be calculated, which has the potential to be a useful diagnostic tool.

8947-33, Session 7

Miniaturized CARS microendoscope probe design for label-free intraoperative imaging

Xu Chen, Xi Wang, Xiaoyun Xu, Jie Cheng, Zhengfan Liu, Seng Weng, Michael J. Thrall, The Methodist Hospital Research Institute (United States); Goh C Alvin, Houston Methodist Hospital (United States); Daniel T McCormick, advanced MEMS (United States); Kelvin Wong, Stephen Wong, The Methodist Hospital Research Institute (United States)

A micro-optics probe for label-free Coherent Anti-Stokes Raman Scattering (CARS) microendoscope at microscopic resolution is presented. The handheld miniature CARS microendoscope probe include custom made micro-electromechanical systems (MEMS) scanning mirror as well as miniature optical and mechanical components. In our design, excitation lasers (pump, stokes beam) from the fiber are collimated and reflects off a reflecting mirror and transmits via a 2D MEMS scanning mirror and a micro-objective subsystem onto the sample; whereas emission light in epi-direction is returned via the same path through the micro-objective subsystem to the fiber and finally collected by a PMT. The exit pupil diameter of collimator is designed to match the diameter of MEMS mirror and the entrance pupil diameter of the micro-objective subsystem. Back aperture diameter of the micro-objective subsystem is designed according to the largest MEMS scanning angle and the distance between MEMS mirror and back aperture. To increase the numerical aperture (NA) of the micro-objective to enhance the signal collecting efficiency, the back aperture diameter of micro-objective is enlarged with achromatic wide angle Keplerian telescope beam expander before excitation light focused onto the sample. The integration of miniaturized micro-optics probe with the full-fiber CARS technology opens up the possibility of in vivo molecular vibration imaging for cancer diagnosis and surgical intervention. Currently, we are applying the CARS probe in radical prostatectomy to detect prostate cancer tissue margins and nerves with in vivo, in situ and less-invasive feature.

8947-34, Session 8

Development of a spectrally-resolved fluorescence tomography system using a NIR swept laser and a digital micromirror array based detection system

Jaedu Cho, Univ. of California, Irvine (United States); Seung Woan Jeon, Chang-Seok Kim, Pusan National Univ. (Korea, Republic of); Orhan Nalcioglu, Gultekin Gulsen, Univ. of California, Irvine (United States)

We present spectrally-resolved fluorescent tomography (FT) small animal imaging system using a broadband NIR swept laser and a digital micromirror array based detection system. To our knowledge, this study demonstrates the first reported spectrally-resolved FT system using a NIR swept laser and a detection system based on a digital micromirror array. On the source side, a compact design of NIR swept laser is employed to excite various fluorophores in tissue. The NIR swept laser consists of a semiconductor optical amplifier, a transmission grating-based wavelength selection filter. The swept wavelength starts from 780 nm to 820 nm, whose center wavelength lies on 800 nm for maximum absorption of Indocyanine Green (ICG). The maximum output power is around 10 mW at the center wavelength. On the detection side, a digital micromirror array, interferometric edge filters, a transmission diffraction grating and a photomultiplier tube comprise the spectrally resolved fluorescent detection system. The interferometric filters block excitation light at the first light collection stage, and then a transmission grating spatially distribute the collected light throughout active micromirror array area at the second stage. Specific bandwidth is selected by spatial modulation of the digital micromirror array to fluorescence emission light. This ultimate combination of spectrally-resolved fluorescence excitation and detection enables visualization of various fluorophores in vivo with high accuracy. To verify our strategy, two distinct but partially overlapping fluorophores are incorporated in a gelatin phantom representing a small animal and their concentration maps are reconstructed with the spectrally-resolved FT system. We are planning to undertake in vivo studies in a couple of months and present the results during the meeting.

8947-35, Session 9

Ultrahigh-throughput imaging flow cytometry using radiofrequency-tagged emission

Eric D. Diebold, Brandon W. Buckley, Bahram Jalali, Univ. of California, Los Angeles (United States)

Fluorescence imaging is the most widely used method for unveiling the molecular composition of biological specimens. However, the weak optical emission of fluorescent probes and the tradeoff between imaging speed and sensitivity is problematic for acquiring blur-free images of fast phenomena, such as sub-millisecond biochemical dynamics in live cells and tissues, and cells flowing at high speed. We present a solution that achieves real-time pixel readout rates of 300 MHz – a 30x improvement as compared to a modern electron-multiplier charge coupled device (EMCCD). Deemed fluorescence imaging using radiofrequency-tagged emission (FIRE), this approach to fluorescence microscopy maps the image into the radiofrequency spectrum using the beating of digitally synthesized optical fields. A single photomultiplier tube (PMT) detects the image fluorescence, and the signal is de-multiplexed using massively parallel digital lock-in amplification to form an image. This work represents a high-speed radiofrequency (RF) communications approach to fluorescence microscopy, which combines the benefits of PMT sensitivity and speed with acousto-optic-based frequency-domain signal multiplexing, RF spectrum digital synthesis, and digital lock-in amplification to enable fluorescence imaging at kHz frame rates. We demonstrate diffraction-limited confocal fluorescence imaging of stationary cells at a frame rate of 4.4 kHz, as well as fluorescence imaging of cells in flow at velocities greater than 1 meter per second. This velocity corresponds to a throughput of approximately 50,000 cells per second, which represents more than an order of magnitude improvement over the current state-of-the-art in fluorescence imaging flow cytometry.

8947-36, Session 9

High-speed flow cytometric analysis of nanoparticle targeting to rare leukemic stem cells in peripheral human blood: preliminary in-vitro studies

Christy L. Cooper, James F. Leary, Purdue Univ. (United States)

Leukemic cancer stem cells are both stem-like and leukemic-like. This complicates their detection as rare circulating tumor cells in peripheral blood of leukemia patients. The leukemic stem cells are also highly resistant to standard chemotherapeutic regimens so new therapeutic strategies need to be designed to kill the leukemic stem cells without killing normal stem cells. In these initial studies we have designed an antibody-targeted and fluorescent (Cy5.5) nanoparticle for targeting these leukemic stem cells and then introducing new strategies for killing them.

Multicolor flow cytometric analyses were performed on a BD FACS Aria III. Human leukemic stem cell-like cell lines MV4-11 (with putative immunophenotype CD123+/CD24-/CD34+/CD38-/ CD10-/Flt-3+) or RS4;11 (with putative immunophenotype CD123+/CD24+/CD38-/ CD10-/Flt-3-) were used as model human leukemic stem cell systems and were spiked into normal human peripheral blood cells containing normal blood stem-progenitor cells (immunophenotype CD123-/ CD34+/CD38-) and Cy5.5-labeled nanoparticles with targeting molecule anti-CD123 antibody. An irrelevant antibody which should not bind to any live leukemic stem cell or normal stem cell was used as a way of distinguishing between true-positive live and false-positive damaged/ dead cells, the latter occurring at much higher frequencies than the very rare (e.g. 0.001 to 0.0001 percent frequency true leukemic stem cells). These studies are designed to measure the targeting sensitivity and specificity of the fluorescent nanoparticles to the putative rare leukemic stem cells with the eventual design to use the nanoparticles to direct killing therapeutic doses to the leukemic stem cells but not to the normal stem-progenitor cells.

8947-38, Session 9

Combining surface sensitive vibrational spectroscopy and fluorescence microscopy to study biological interfaces

Chi Zhang, Joshua Jasensky, Jing Wu, Zhan Chen, Univ. of Michigan (United States)

We developed a novel system by combining a surface sensitive vibrational spectroscopic technique, sum frequency generation (SFG) and total internal reflection fluorescence (TIRF) microscopy, for biological interfacial studies. A compact microscope was built together with a special platform on a commercial SFG spectrometer. SFG is a second-order nonlinear optical spectroscopic technique that can provide vibrational spectra of interfaces with sub-monolayer sensitivity. We utilized the evanescent wave of each input beams to achieve total internal reflection (TIR)-SFG for biological interfacial studies by probing only the cell interface closest to the substrate, without the confusion from other interfaces from the sample. With the help of the microscope, the laser beam focal spot in our TIR-SFG experiment can be traced that allows for studies on heterogeneous samples. Furthermore, utilizing the 532 nm visible beam from SFG or tunable visible beam generated from the optical parametric amplifier (OPA), TIRF microscopy with multiple excitation channels can be achieved. TIRF signals are collected using the microscope and detected using an electron multiplying charge-coupled device (EMCCD), which provides single molecule detection capability. Interfacial molecular structural information of biological interfaces can be revealed by studying intrinsic molecular vibrational peaks using SFG spectroscopy while the interfacial molecular dynamics or activities can be imaged using TIRF microscopy. This technique provides a unique way to study molecular structures of biological interfaces and can provide a visualization of interfacial mechanisms/activities. This research paves the way for the possible applications of biological interfacial studies such as cell adhesion, marine biofouling, and material biocompatibility.

8947-39, Session 9

10?10-pixel 606kS/s multipoint fluorescence correlation spectroscopy CMOS image sensor

Keiichiro Kagawa, Taishi Takasawa, Bo Zhang, Min-Woong Seo, Kaita Imai, Shizuoka Univ. (Japan); Jotaro Yamamoto, Masataka Kinjo, Hokkaido Univ. (Japan); Susumu Terakawa M.D., Hamamatsu Univ. School of Medicine (Japan); Keita Yasutomi, Shoji Kawahito, Shizuoka Univ. (Japan)

To observe molecular transport in a living cell, a high-speed CMOS image sensor for multi-point fluorescence correlation spectroscopy is developed. To achieve low-noise and high-speed simultaneously, a prototype CMOS image sensor is designed based on complete pixel-parallel and multi-channel pipelined pixel readout. The prototype chip fabricated in 0.18-?m CMOS image sensor technology has 16?10 pixels including 10?10 effective pixels, 4?10 optical black pixels, and 2?10 test pixels. The pixel pitch and the photosensitive area are 56?m and 10?m in diameter without a microlens, respectively. The pixel has one photodiode and three sets of a charge detection node and a source follower amplifier. Every source follower is connected to a correlated double sampling (CDS) amplifier with a large gain to reduce both of reset noise at the charge detection node and the noise from the source follower. Thus, the prototype chip is equipped with 480 CDS amplifiers for 160 pixels in total. The amplified signals in the same column are multiplexed, and they are read out through 16 analog outputs. The output signals are converted to digital ones with 16 analog-to-digital converters with a 12-bit resolution. Then, they are continuously transferred to a personal computer through CameraLink in the medium configuration. In the experiment, each of the three channels is operated at 202kS/s, so that the total sampling rate of

606kS/s is achieved. The measured average random noise is 32.7LSB, which is equivalent to 3 electrons.

8947-40, Session 9

TCSPC based approaches for multiparameter detection in living cells

Karolina Jahn, Univ. of Potsdam (Germany); Volker Buschmann, Felix Koberling, PicoQuant GmbH (Germany); Carsten Hille, Univ. of Potsdam (Germany)

In living cells a manifold of processes take place simultaneously. This implies a precise regulation of intracellular ion homeostasis. In order to understand their spatio-temporal pattern comprehensively, the development of multiplexing concepts is essential.

Due to the multidimensional characteristics of fluorescence dyes (absorption and emission spectra, decay time, anisotropy), the highly sensitive and non-invasive fluorescence microscopy is a versatile tool for realising multiplexing concepts. A prerequisite are analyte-specific fluorescence dyes with low cross-sensitivity to other dyes and analytes, respectively. Here, two approaches for multiparameter detection in living cells are presented.

Insect salivary glands are well characterised secretory active tissues which were used as model systems to evaluate multiplexing concepts. Salivary glands secrete a KCl-rich or NaCl-rich fluid upon stimulation which is mainly regulated by intracellular Ca2+ as second messenger. Thus, pairwise detection of intracellular Na+, Cl- and Ca2+ with the fluorescent dyes ANG-2, MQAE and ACR were tested. Therefore, the dyes were excited simultaneously (2-photon excitation) and their corresponding fluorescence decay times were recorded within two spectral ranges using time-correlated single-photon counting (TCSPC).

A second approach presented here is based on a new TCSPC-platform covering decay time detection from picoseconds to milliseconds. Thereby, nanosecond decaying cellular fluorescence and microsecond decaying phosphorescence of Ruthenium-complexes, which is quenched by oxygen, were recorded simultaneously.

In both cases changes in luminescence decay times can be linked to changes in analyte concentrations. In consequence of simultaneous excitation as well as detection, it is possible to get a deeper insight into spatio-temporal pattern in living tissues.

8947-41, Session 10

Nanoscale disorder of biological samples mapped by whole-slide spectral microscopy

John E. Chandler, Hariharan Subramanian, Khushi Vyas, Lusik Cherkezyan, Vadim Backman, Northwestern Univ. (United States)

Partial Wave Spectroscopic Microscopy (PWS) is a spectroscopic, light-scattering microscopy technique which enables quantification of nanoscale structural disorder within an inhomogeneous medium. PWS has shown significant clinical potential as a cancer screening modality with previous experiments showing PWS detects intracellular nanoarchitectural changes associated with the field effect of carcinogenesis from ~30 discrete measurements of individual cells from a homogeneous population for major cancers including: lung, colon, ovarian, and esophageal. To expand the capabilities of PWS to samples consisting of heterogeneous cell populations as in tissue sections and enable mapping of the nanoscale disorder for the entire sample, we have modified the instrumentation and analysis to enable collection of large continuous PWS images in the manner of whole-slide imaging systems. A new analysis algorithm has been developed that requires 8% (540-560nm) of the previous spectral range (450-700nm) and up to 10X fewer pixels per cell. On a prior lung cancer dataset this new algorithm compared favorably with analysis of the entire spectrum and pixels with both methods generating an effect size of approximately 136. A PWS image of an entire prostate tissue section was collected by tiling together spectral data cubes (x,y,?) collected in less than 1 second per spatial position from 540-560nm with binning of the images for reduced file-size and speed. This measurement yielded a continuous map of the nanoscale disorder of the entire tissue specimen which can be used to form a quantitative diagnosis and can be compared directly to a traditional histology image of the same area.

8947-42, Session 10

High-speed focal modulation microscopy for calcium imaging in thick tissues

Shilpa Pant, Nanguang Chen, National Univ. of Singapore (Singapore)

Focal Modulation microscopy (FMM) is a novel technique that can be used for deep imaging in optically thick tissues with single photon excited fluorescence. FMM makes use of a spatial light modulator to phase modulate half of the extent of the excitation light beam. Superposition of the two half beams at the focal point gives rise to intensity modulated excitation and emission light. FMM uses the principle that scattered excitation photons will not contribute to the modulated emissions and thus by filtering out the DC component from the whole signal, it is possible to remove the contribution of scattered excitation photons. This causes a drastic improvement in the signal-to-background ratio. A penetration depth of 600 µm has been demonstrated with this technique. We are currently working on improving the speed of image acquisition. For this, we have developed an image-scanning system in which the excitation light is scanned across the sample along a line. The confocal fluorescence emissions or back-scattered light is then scanned across the face of a 2D CCD camera to form the image. Our current system can be used at 40 fps for an image size of 696x520 pixels. Faster imaging speed of upto 100 fps can be achieved with smaller image sizes. A greater penetration depth compared to a confocal system, accompanied with higher acquisition speed is demonstrated with in vivo time-lapse calcium imaging studies of neurogenesis during development in zebrafish.

8947-43, Session 10

The use of fluorescence fluctuation in polarization sensitive experiments

Dror Fixler, Bar-Ilan Univ. (Israel)

In fluorescence fluctuation polarization sensitive experiments, the limitations associated with detecting the rotational timescale are usually eliminated by applying fluorescence correlation spectroscopy analysis. A new method to extract the rotational correlation time of molecule in fluorescence fluctuation polarization sensitive measurements is suggested in our talk. This new method is advantageous in cases where the rotational correlation time of the fluorescent molecule is much lower than the temporal resolution of the system, or in cases where the rotational correlation time is not observed by standard autocorrelation analysis methods such as fluorescence correlation spectroscopy (FCS) or rotational correlation functions analysis. Very short rotational correlation times within the range of the anti-bunching time (1-10 ns) of the fluorescent molecules in different viscosities of the medium were then extracted using this method. The methods that we will describe in our talk are suggesting a new stochastic analysis and experimental treatment of fluorescence fluctuations as a result of different limitations of the measurement system or as a result of physical processes such as rotational diffusion. Eventually, these new methods will make it possible to extract more accurate and efficient information on the molecules and their environment while observing different fluorescent samples or living cells, especially in low emitted fluorescence intensity and even on the single fluorescent molecule level.

8947-44, Session 11

Bacterial response to confinement in microfluidics

Ben Libberton, Univ. of Liverpool (United Kingdom); Falco C. M. van Delft, Philips Research Nederland B.V. (Netherlands); Dan V. Nicolau, Univ. of Liverpool (United Kingdom) and McGill Univ. (Canada)

No Abstract Available

8947-45, Session 11

Quantitative, noninvasive, optical biomarkers of altered mitochondrial organization in precancerous tissues

Irene Georgakoudi, Joanna Xylas, Antonio Varone, Tufts Univ. (United States); Kyle P. Quinn, Tufts Univ (United States); Margaret McLaughlin-Drubin, Karl Munger, Brigham and Women's Hospital (United States)

Mitochondrial organization is often altered to accommodate specific energy-related functions. Alterations in metabolism are a hallmark of cancer; thus, the interdependence between mitochondrial function and organization could be exploited to improve detection and understanding of cancer progression. In this study, we present a novel, quantitative method to assess mitochondrial organization in three-dimensional tissues using exclusively endogenous fluorescence. We examine the overall and depth-dependent organization of mitochondria within fully differentiated engineered epithelial tissue equivalents (EETEs) consisting of human foreskin keratinocytes (HFKs). We compare them to EETEs that contain HFKs that were immortalized following expression of the full high-risk HPV16 viral genome and exhibit loss of differentiation and a phenotype similar to that of cervical pre-cancers. In addition, we characterize EETEs constructed with HFKs that express the high-risk HPV16 oncoproteins E6 and E7, which inhibit the p53 and pRB tumor suppressor pathways, respectively, that are mutated in many human cancers. Thus, these EETEs may also model non-HPV associated pre-cancers. We identify unique patterns of mitochondrial organization in each of the EETE types we examine, and we demonstrate that they provide complementary, and potentially more sensitive, organizational information when compared to traditional morphological pre-cancerous hallmarks related to larger features, such as cell nuclei. Finally, we present evidence that these mitochondrial organization biomarkers are relevant for the characterization of normal and pre-cancerous ex vivo human cervical tissue specimens, thus illustrating the potential for translation as a useful diagnostic or treatment-monitoring tool.

8947-46, Session 11

3D manipulation and visualization of in-vitro cells by optical tweezers and digital holographic microscopy

Pietro Ferraro, Francesco Merola, Lisa Miccio, Istituto Nazionale di Ottica (Italy); Pasquale Memmolo, Istituto Nazionale di Ottica (Italy) and Ctr. for Advanced Biomaterials for Health Care, CRIB (Italy); Paolo A. Netti, Univ. degli Studi di Napoli Federico II (Italy); Giuseppe Coppola, Istituto per la Microelettronica e Microsistemi (Italy); Giuseppe Di Caprio, Rowland Institute at Harvard (United States)

Digital holographic microscopy is a powerful tool for imaging micro-objects contained into a three dimensional (3D) volume. Cells display a very singular behavior when seeded in 3D matrices like collagen, fibrin or cell derived matrix. In fact, cells scan the surrounding environment and highly dynamic membrane processes are continuously projected out the cell body in order to eventually forming new adhesions. Such a dynamic morphology might hamper the estimation of the cell dimensions as well as the assessment of its real position in the 3D volume. In particular, the correct estimation of the position, through a typical 3D holographic tracking, is highly influenced by morphological variations. Therefore, we propose to use the quantitative phase-contrast map, obtained from the digital holograms of cells recorded in microscope configuration, in order to investigate 3D positions and 3D morphological changes together.

Moreover, we show that it is possible to trap cells (fibroblasts and bovine spermatozoa) by using optical tweezers, and to place them in desired positions, suitable for holographic recordings. Even in these cases we recover the 3D shape of the cells starting from the quantitative phase maps (QPMs).

8947-47, Session 11

Time-gated imaging of near-infrared quantum dots for in vivo cell tracking

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In vivo cell tracking is a promising tool to improve our understanding of certain biological processes such as circulating tumor cells migration or immune cells activity. Several cells tracking techniques have been developed like MRI or PET but remain ill adapted to detect rare and individual cells because of their low spatial resolution. Fluorescence detection is a promising alternative. Its sensitivity is however limited by the high tissue autofluorescence and poor visible light penetration depth. To overcome these limitations, we have developed a novel cell imaging modality, based on time-gated wide field fluorescence imaging of long lifetime near-infrared quantum dots (QDs).

We will report the synthesis and characterization of Zn-Cu-In-Se / ZnS (core/shell) QDs composed of low toxicity materials. These QDs exhibit a bright emission centered around 800 nm, where absorption and scattering of tissues are minimal. They are coated with a new home-made surface chemistry, which yields to small, stable and bright individual probes into live cell cytoplasm, even several days after the labeling.

Thanks to a fluorescence lifetime much longer (150-200 ns) than tissues autofluorescence (5-10 ns), and a time-resolved detection, QDs emission can be efficiently discriminated from autofluorescence. This leads to an important increase of sensitivity that allows in vivo tracking of circulating cells.

We will present the wide field time-gated fluorescence microscope we developed and show preliminary results on detection of individual circulating near-infrared QDs stained cells.

8947-48, Session 11

Quantitative sensing of microviscosity in protocells and amyloid materials using fluorescence lifetime imaging of molecular rotors

Alex J. Thompson, Imperial College London (United Kingdom); Dora T.-Y. Tang, Univ. of Bristol (United Kingdom); Therese W. Herling, Univ. of Cambridge (United Kingdom); Rohaida C. Che Hak, Stephen Mann, Univ. of Bristol (United Kingdom); Tuomas

P. J. Knowles, Univ. of Cambridge (United Kingdom); Marina K. Kuimova, Imperial College London (United Kingdom)

There is significant interest in the development of techniques that permit quantitative measurement of viscosity on the microscopic scale. Molecular rotors (MRs) are fluorophores that have a fluorescence quantum yield that is dependent upon intermolecular rotation. Thus, the fluorescence lifetime of MRs varies as a function of viscosity with high viscosities inhibiting intermolecular rotation and increasing the fluorescence lifetime. Here, we present the use of time-resolved fluorescence spectroscopy (TRFS) and fluorescence lifetime imaging microscopy (FLIM) to measure the fluorescence lifetimes of MRs in two different biological systems.

Using FLIM with 2-photon excitation, we investigated a novel design for a so-called 'protocell', consisting of a polyelectrolyte (PDDA-ATP) core encapsulated by an oleic acid membrane. Protocells – models for precursors to the modern cell – are of interest in studies of early cellular evolution. We measured the viscosity in the core and membrane regions of the novel protocell with two different MRs (kiton red and BODIPY respectively) and studied the formation and stability of the protocells under various conditions.

Secondly, the formation of an amyloid material was studied using the MR Cy3. Amyloid materials are aggregated proteins and are most commonly studied because of their relevance to neurodegenerative diseases such as Alzheimer's. Using FLIM of MRs we have quantitatively measured viscosity over the course of protein aggregations. Furthermore, using phasor analysis, we can differentiate between lifetime variations caused by viscosity changes and by binding of the fluorophore to the protein. Neither of the above is possible with other methods used to monitor protein aggregation.

8947-49, Session 11

Asymmetric-detection time-stretch optical microscopy (ATOM) for high-contrast and high-speed microfluidic cellular imaging

Terence T. W. Wong, Andy K. S. Lau, Matthew Y. H. Tang, Kenneth K. Y. Ho, Kenneth K. Y. Wong, Anderson H. C. Shum, Kevin K. Tsia, The Univ. of Hong Kong (Hong Kong, China)

Time-stretch microscopy has recently been demonstrated as a powerful tool for achieving high-speed cellular imaging with ultra-high frame rate (> MHz). This new imaging modality is particularly invaluable for high-throughput biomedical diagnostic which requires ultrafast imagers to capture unambiguous images of individual flowing cells in a large population for high-precision statistical analysis or even enabling single-cell analysis. Prior demonstration of using time-stretch imaging for high-throughput microparticle analysis operated in the telecommunication band – a wavelength band which is not fully compatible with the main stream of biophotonics, and also results in relatively low diffraction-limited resolution. The reported time-stretch image quality so far is thus inadequate for fully utilizing the advantage of this technology for realizing "image-based" flow cytometry.

In this regard, we report a further advancement in time-stretch microscopy, coined as asymmetric-detection time-stretch optical microscopy (ATOM), for ultrafast contrast-enhanced cellular imaging in flow. We enable high-resolution ATOM operation in the 1μm wavelength regime which is favorable for biomedical applications. Moreover, the system incorporates a simple asymmetric detection scheme which enhances the label-free time-stretch image contrast through accessing the phase-gradient information. We demonstrate ultrafast ATOM of biological cells flowing in a polydimethylsiloxane microfluidic channel which is custom-designed for accommodating an objective lens with high numerical aperture in order to achieve higher image resolution. The system is capable of capturing and differentiating high-contrast label-free images of different cell types (HeLa and hepatocyte carcinoma) flowing at a record high speed of 4.7m/s – an imaging throughput equivalent to ~100,000 cells/sec without image blur.

8947-50, Session 12

Multispectral sorter for rapid, nondestructive optical bioprospecting for algae biofuels

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Microalgal biotechnology is a nascent yet burgeoning field for developing the next generation of sustainable feeds, fuels, and specialty chemicals. Among the issues facing the algae bioproducts industry, the lack of efficient means of cultivar screening and phenotype selection represents a critical hurdle for rapid development and diversification. To address this challenge, we have developed a multi-modal and label-free optical tool which simultaneously assesses the photosynthetic productivity and biochemical composition of single microalgal cells, and provides a means for actively sorting attractive specimen (bioprospecting) based on the spectral readout. The device integrates laser-trapping Raman spectroscopy and pulse-amplitute-modulated (PAM) fluorometry of microalgal cells in a flow cell. Specifically, the instrument employs a dual-purpose epi-configured IR laser for single-cell trapping and Raman spectroscopy, and a high-intensity visible wavelength trans-illumination LED bank for detection of variable photosystem II fluorescence. Near IR Raman scatter of single algae cells revealed vibrational modes corresponding to the speciation and total lipid content, as well as other major biochemical pools, including total protein, carbohydrates, and carotenoids. PSII fluorescence dynamics provide a quantitative estimate of maximum photosynthetic efficiency and photochemical and non-photochemical quenching processes. The combined spectroscopic readouts provide a set of metrics for subsequent optical sorting of the cells by the laser trap for desirable biomass properties, e.g. the combination of high lipid productivity and high photosynthetic yield. In summary, the device provides the means for rapid and non-invasive evaluation and sorting of promising parent cells from algae cultures and environmental samples for biofuels applications.

8947-51, Session 12

Lens-free digital in-line holographic imaging for wide field-of-view, high-resolution and real-time monitoring of complex microscopic objects

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Lens-free Holographic Microscopy (LHM) holds great promise for many biomedical and industrial applications due to its conceptual simplicity. However, the challenge lies in achieving image quality comparable to conventional microscopes. One of the main factors reducing image quality in LHM is the well-known twin-image problem. A variety of twin-image suppression techniques exist but are not suitable for real-time monitoring of complex microscopic structures.

We demonstrate a high-throughput LHM system deployable in biomedical and industrial applications. The system consists of a multi-wavelength fiber-optics-based coherent light source and a monochrome 10Mp imager with 1.67μm pixel pitch. This system resolves 1.23μm-thin lines on the USAF 1951 optical target while the full field-of-view is approx. 27mm2. The system reaches throughput of up to 50 fps in

full-HD resolution. We use the in-line lens-free imaging configuration to achieve maximum field-of-view and high resolution at minimal hardware cost. Our main contribution is a unique iterative-phase-retrieval method based on multi-wavelength image acquisition. This technique allows us to overcome the inherent deficiencies of the in-line holography (i.e. the twin-image problem). To complement the imaging hardware, we have implemented a GPU-based real-time post-processing software, consisting of automated object identification and segmentation, object-based autofocus and multi-wavelength iterative phase retrieval. We have evaluated our system in a number of experiments ranging from thin-film industrial inspection to in-vitro imaging of large biological structures (e.g. Ø 2-3mm dense stem-cell colonies). In biomedical applications, our system reaches single-cell to sub-cellular resolution in intensity as well as in phase images.

8947-52, Session 12

Plasmonic field localization by subwavelength metallic nanoaperture arrays for imaging biomolecular movement

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Recently, super-resolution imaging techniques such as STED, PALM, and STORM have produced tremendous interests for direct observation of biomolecules in intracellular and extracellular environment. With a nanometer scale image resolution, many approaches have been attempted to explore molecular characteristics of biological targets of diverse nature. In this presentation, we investigate surface plasmon based electromagnetic field localization to break the diffraction limit of conventional fluorescence microscopy by creating dramatically amplified hot spots at subwavelength metallic nanoaperture surface. We explore locally amplified excitation and subdiffraction-limited sampling of target fluorescence to induce effective enhancement of imaging resolution. In this study, we used periodic arrays of optical nanoapertures for plasmonic field localization. Nanoapertures of different sizes and shapes were designed to produce hot spots of different characteristics. The design process was performed numerically based on rigorous coupled wave analysis. The arrays of nanoholes or nanoposts were fabricated on a 50-nm thick gold film surface using electron beam lithography. For optical imaging experiments, the fabricated nanoaperture arrays were mounted in an optical fluorescence microscope and used to image Cy3-labelled bacteria (Mycoplasma Mobile: M. Mobile) or gliding microtubules. By experimentally testing gliding microtubules, effective resolution on the order of 70 nm has been validated. The results also indicate the feasibility of improving imaging resolution both in the lateral and axial direction to elucidate, for example, axial displacement and positional variations of a single M. Mobile as its movement was reconstructed with a nanoscale resolution. We expect the approach to prove useful for more diverse biological targets.

8947-53, Session 12

Video lensfree microscopy of 2D and 3D culture of cells

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Research is continuously developing imaging methods to better understand the function of biological systems at microscopic scale. Advanced experiments performed with e.g. confocal microscopy are very effective at observing cellular and sub-cellular level but miss the full picture where the environment predominates. As an alternative, we are developing lensfree video microscopy that opens new range of capabilities. Lensfree video microscopy allows watching culture of cells straight into the incubator over very large field of view (25 and 44 mm2) and extended period of times: comprehensive data can be gathered with huge statistics, both in space and time. At first, we present several examples amongst a total of 1000 hours acquisitions to demonstrate that this simple technique can capture cells in culture within different physical environment. Namely we observed several cell lines (kidney epithelial cells, RWPE1, primary human fibroblasts, PC3, HUVEC endothelial, etc.) cultured with different recipients (35 mm Petri dish, 8 wells plate, etc.) over distinct substrates (polylisin, matrigel, PniPam, inside extra-cellular matrix, etc.). Next, we emphasize on three different case studies: the effect of drugs on prostatic cells culture, the quantitative analysis of endothelial cells network formation, and by coupling lensfree microscopy with 3D cells culture, the study of epithelial tissue morphogenesis and differentiation. In sum we demonstrate that lensfree video microscopy is a powerful tool to conduct cell in culture experiments. In combination with 3D assays, it allows to probe models that mimic functions of living tissues. Applications are in the realm of fundamental research and high-throughput screening.

8947-54, Session 12

On measuring cell confluence in phase contrast microscopy

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Understanding the mechanisms behind the proliferation of Mesenchymal Stem cells and neurons can lead to a greater insight of the behaviour of these cells throughout their life cycles. Traditional methods of measuring cell confluence (i.e. the percentage of the growth surface covered by cells) involve inherently subjective estimation. Here, measuring cell growth and (thus) assessing cell quality in monolayer cultures are usually achieved using standard imaging techniques such as phase contract microscopy which monitors several parameters simultaneously. However, most current computational methods have been developed for fluorescent imaging primarily for ease of segmentation. This paper investigate an objective approach to measure cell confluence using live cell imaging techniques coupled with integrated incubator platform. Building on the earlier work of Bradhurst et al. which made use of the change of pixel intensity gradient to localise individual, mature, cells and better define their shape, our algorithm takes into account the bright double haloed cells that indicate mitosis, and neuron cell bodies in order to generate a more realistic estimation of the confluence measure. The results obtained are analysed and compared with the method presented by Bradhurst et al., a subjective score based on guesstimation, and, ultimately, an actual score found by manual segmentation. Early results show that the method presented is a practical candidate for real time analysis achieving greater accuracy when compared to the other approaches.

8947-55, Session 12

Towards optical cell transfection inside a micro flow cell

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Optical transfection of cells has been shown to be an extremely effective and efficient method which ensures high cell viability. For optical transfection cells are subjected to intense, focused laser radiation which leads to a temporary opening in the cell membrane. However, addressing single cells with laser pulses is a tedious and slow approach. We present first measurements aiming at an experimental setup which is suitable for high throughput and automated optical cell transfection. In our setup, cells flow through a micro-flow tube where they are spatially confined. The laser radiation is focused into the flow tube in a way that an elongated focal region is realized. This makes the time consuming aiming of the laser beam at individual cells unnecessary and opens the possibility to develop a completely automated system. The elongated laser focal region is realized by a quasi-Bessel beam which is generated by an axicon-lens setup. We present test measurements of the newly employed setup and discuss its suitability to be fully integrated into a flow cell sequencing system.

8947-56, Session 13

Fast, 3D imaging via confocal line scanning of a Bessel beam using a single galvo mirror

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Light-sheet microscopy is becoming increasingly popular in recent years because of its inherent z-sectioning and reduced photobleaching and photodamage compared to epi-excitation. While light-sheet excitation was initially performed by focusing a Gaussian beam by a cylindrical lens into a thin sheet of light, diffraction of the Gaussian beam made this technique difficult to implement for small beam waists (e.g. < microns) over large imaging areas (tens of microns). To circumvent issues related to diffraction, a number of laboratories (most notably Betzig et al) have turned to scanning a Bessel beam for excitation in light-sheet microcopy, enabling a simultaneous diffraction-limited excitation and large field of view. However, the side lobes of the Bessel beam can generate out-of-focus background. This background can be reduced either by two-photon excitation or by structural illumination methods that require acquisition of multiple frames of images, two time or equipment expensive techniques. An alternative approach is to de-scan the fluorescence by a second galvo-mirror and then to spatially filter this background. However, synchronization of multiple galvo-mirrors is required. Here we report a light-sheet microscopy system that can perform Bessel beam scanning and spatial filtering with only a single galvo-mirror. The same galvo that scans the excitation beam also de-scans the emitted fluorescence through a slit, such that the fluorescent image is reconstructed in real-time. Compared to two-photon Bessel beam excitation or other confocal line scanning approaches, our method is of lower cost, lower maintenance, and doesn't require calibration and synchronization of multiple galvo mirrors.

8947-57, Session 13

A new 3D tracking method for cell mechanics investigation exploiting the capabilities of digital holography in microscopy

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A method for 3D tracking has been developed exploiting Digital Holography features in Microscopy (DHM). In the framework of self-consistent platform for manipulation and measurement of biological specimen we use DHM for quantitative and completely label free analysis of samples with low amplitude contrast. Tracking capability extend the potentiality of DHM allowing to monitor the motion of appropriate probes and correlate it with sample properties. Complete 3D tracking has been obtained for the probes avoiding the amplitude refocusing in traditional tracking processes.

Moreover, in biology and biomedical research fields one of the main topic is the understanding of morphology and mechanics of cells and microorganisms. Biological samples present low amplitude contrast that limits the information that can be retrieved through optical bright-field microscope measurements. The main effect on light propagating in such objects is in phase. This is known as phase-retardation or phase-shift. DHM is an innovative and alternative approach in microscopy, it's a good candidate for no-invasive and complete specimen analysis because its main characteristic is the possibility to discern between intensity and phase information performing quantitative mapping of the Optical Path Length.

In this paper, the flexibility of DH is employed to analyze cell mechanics of unstained cells subjected to appropriate stimuli. DHM is used to measure all the parameters useful to understand the deformations induced by external and controlled stresses on in-vitro cells. Our configuration allows 3D tracking of micro-particles and, simultaneously, furnish quantitative phase-contrast maps. Experimental results are presented and discussed for in vitro cells.

8947-58, Session 13

Raman tweezers in microfluidic systems for analysis and sorting of living cells

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We develop systems combining Raman spectroscopy and optical trapping in microfluidic environment. These systems should serve for Raman analysis and separation of the selected objects (living cells) by means of optical tweezers. We have designed special microfluidic chips, where the examined cells are propelled by gravity and sorted by entrapment by optical tweezers with concurrent motorized motion of the microscopic stage. The cells are loaded on the top of a liquid medium in a wide vertical microchannel. As the cells are sinking, they enter an area with optical trap, adjacent to a narrow horizontal channel branching off the main one. The cells are recognized by image analysis, the optical trap is positioned to trap the cell, the cell is analyzed by Raman spectroscopy, and either dropped, or moved to the horizontal channel. Single laser of 785nm wavelength is used both for trapping and Raman analysis. After all the cells in the vertical channel settle, the selected cells can be sucked out of the chip and transplanted to a suitable medium. The Raman analysis uses rolling circle filter algorithm for on-line filtration, and intensity comparison in user-defined spectral regions. If the given ratio reaches over a selected threshold, the cell is separated, otherwise it is discarded. This analytical and sorting system has the benefit of high robustness, even unfiltered samples of unicellular organisms, e.g. algae, can be successfully sorted. Our systems are continuously being developed to optimize the transfer of the few selected cells to the cultivation medium after the selection.

8947-60, Session 13

Photodynamic therapy for treatment of chromoblastomycosis

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The chromoblastomycosis is a disease caused mainly by the infection of the fungus Fonsecaea pedrosoi, that affects the skin, subcutaneous tissue and, in a few cases, can reach internal organs that presents. This is a public health problem in some countries of Latin America and countries with tropical or subtropical climates. The current treatment for this disease is inefficient and long-term. In this context, is necessary the improvement of new technologies. Photodynamic Therapy (PDT) is a relatively new technique which is a combination of drug, light and oxygen leading to the production of oxidative cytotoxic agents, leading the death of pathogen. The aim of this research is evaluate the effect of PDT with 5-aminolevulinic acid 5% in the F. pedrosoi. The fungus was incubated with ALA 5% with two concentration of dimethyl sulfoxide (DMSO) 1% and 2% by 20, 40, 60, 120, 180, and 360 minutes. Confocal imaging showed Protoporphyrin (PpIX) fluorescence in the hypha: 20 minutes after, it was observed PpIX in the superficial region and 1, 2 and 3 hours after the photosensitizer was located in hyphas deeper, more spread with the better result testing 2% DMSO and 3 hours of incubation. In the next step we will initiate PDT application at different dose and irradiance. In conclusion, the F. pedrosoi is able to produce PpIX and therefore, it is possible to apply different protocols of PDT.

8947-61, Session 13

Optical properties of red blood cells: an optical tweezer based analysis

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We have measured a birefringence induced retardation of 9nm at the centre of a normal human Red Blood Cells (RBC) using a polarizing microscope. The birefringence is measurable only in the dimple region of the RBC. We calculate the birefringence due to the phospholipid content of the RBC membrane using a simple geometric model for the RBC surface assuming that the molecules are oriented normally to the local surface curvature. Taking into account the variation in orientation of the phospholipid molecules, our calculations predict a value of birefringence in the rim region of the RBC to be approximately half of that in the dimple region in agreement with our measurements.

As a consequence of its birefringence, an RBC in a linearly polarized optical tweezer is trapped with its diameter parallel to the plane of vibration of the light. We find that the RBC follows a slow rotation of the linear polarizer and that an estimate of its birefringence made by measuring the time taken for realignment of the cell agrees well with that measured using the polarizing microscope. However, circularly polarized light fails to generate consistent rotations which we attribute to very low value of retardation in a human RBC resulting in torques that are comparable in magnitude to random thermal torques.

8947-84, Session 13

Viscoelastic property of the cell membrane influenced by the adhesion of macrophages on the matrix extracellular

Samuel T. Souza, Lais C. Agra, Emiliano Barreto, Jandir M. Hickmann, Univ. Federal de Alagoas (Brazil); Eduardo J. S. Fonseca, Univ Federal de Alagoas (Brazil)

Macrophages have important roles in the immune response and tissue homeostasis. Its capacity for phagocytosis renders them effective at patrolling through a variety of tissues. In addition, the hallmarks of activation of macrophages can be verified by cell adhesion and spreading on extracellular matrix (ECM). Among the ECM components, fibronectin has been recognized as the key element in promoting cell adhesion and various functions of macrophages. However, yet is not fully understood if the deformation capacity of the membrane plasmatic is influenced by cell adhesion on the ECM.

In this work we studied viscoelastic properties of the macrophages membrane adhered on a film of fibronectin using the Atomic Force Microscopy (AFM) aiming to identify possible interference of extracellular matrix on the biomechanical characteristics of cells. To this study, J774 macrophages were incubated for 1h and 48h on glass coverslips and on glass coverslips recovered with EMC. After 1h or 48h of incubation, cells were fixed and analyzed with the AFM nanoindentation technique.

Ours results have revealed that no significant variation in Young's modulus of cells incubated just on glass coverslips for 1h and 48h. However, they showed the increase in the Young's modulus of about 36% and 50% for cells incubated on glass coverslips recovered with EMC, respectively, as compared with cells incubated just on glass coverslip for 1 and 48h.

In summary, ours results indicate that cell adhesion on fibronectin induce a significant alterations on viscoelastic properties of the cell membrane, suggesting involvement of the intracellular components, such as cytoskeleton, triggered by interaction between cell and ECM.

8947-62, Session 14

Three-dimensional morphological imaging of human induced pluripotent stem cells by using low-coherence quantitative phase microscopy

Toyohiko Yamauchi, Yumi Kakuno, Kentaro Goto, Tadashi Fukami, Norikazu Sugiyama, Hidenao Iwai, Yoshinori Mizuguchi, Yutaka Yamashita, Hamamatsu Photonics K.K. (Japan)

There is an increasing need for non-invasive imaging techniques in the field of stem cell research. Label-free techniques are best fit for assessment of stem cells because the cells remain intact after imaging, and they can be used for further studies such as those for differentiation induction. To develop a high-resolution label-free imaging system, we have been working on a low-coherence quantitative phase microscope (LC-QPM). LC-QPM is a Linnik-type interference microscope equipped with a nanometer-resolution optical-path-length control. The lateral and vertical resolutions of our system are 0.5 and 0.93 μm, respectively, and this performance makes it possible to capture sub-cellular morphological features of live cells without labeling. By using LC-QPM, we reported on three-dimensional imaging of membrane fluctuation, dynamics of filopodia, and motions of intracellular organelles. In this presentation, we report three-dimensional morphological imaging of human induced pluripotent stem cells (hiPS cells). Two groups of monolayer hiPS cell cultures were prepared; one group was cultured in a suitable culture medium that kept the cells undifferentiated, and the other group was cultured in a medium supplemented with retinoic acid, which forces the stem cells to differentiate. The three-dimensional images of the cells

in the 2 groups show distinctive differences, especially with respect to surface roughness. We expect that our LC-QPM system will be further applied to assess many other conditions of stem cells.

8947-63, Session 14

Image Informatics for Studying Signal Transduction in Cells Interacting with 3D Matrices

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Image informatics are imaging-based approaches that infer the role of individual components of a signaling pathway based on how particular perturbations to the pathway affect cell response (quantified based on imaging assays) to particular stimuli. So far, image informatics have been applied in studies of signaling in cells cultured on flat plastic surfaces. However, it is known that cell response depends on its surrounding microenvironment. This study extends the image informatics approach in signaling studies in cells interacting with a biologically relevant 3D insoluble environment (matrix: biomaterials or tissue ECM) using lentiviral shRNA protein knock-down and spectral multi-photon imaging. The response of the cell-matrix system is described by quantifying the statistics of morphology, protein expression, and immediate microenvironment (cell-matrix and cell-cell interactions) of single cells. Results from several treatment conditions (TC; each corresponding to a particular stimulus and system perturbation) are then compared in order to infer information on pathway components based on TC similarity patterns. The developed methodology is applied in a pilot study of TGF? signalling via the SMAD pathway in fibroblasts seeded inside porous collagen scaffolds (a system relevant to myofibroblast differentiation during wound healing). Preliminary statistical analysis suggests that although SMAD2 and SMAD3 proteins are known to be the “canonical” carriers of TGF?1 signals, differential effects of isoforms TGF?1 and TGF?3 to cells could be attributed to the “non-canonical” SMAD1 and SMAD5.

8947-64, Session 14

Automated 3D laser printing of cells and biomaterials for tissue engineering

Florent Deloison, Helen Desrus, ALPhANOV (France); Muhammad Ali, Fabien Guillemot, TEAL: Tissue Engineering Assisted by Laser (France)

Tissue engineering aims at partial or complete regeneration of deficient parts of the body. Stem cells can be taken from patients, specialized in the cell type needed and printed ex situ or in situ to perform an autologous graft or to directly fix the pathological part. In the short term, automation of the fabrication process may allow up-scaling the production of samples with good reproducibility for fundamental research, cosmetic or pharmacological trials. One method to print such tissues is the bottom-up approach: In order to give its form and its function to the tissue, cells must be organized and put together following a specific pattern. This can be directly done at the cell scale thanks to the LIFT technique (Laser Induced Forward Transfer). We’ve demonstrated that it was possible to throw and drop off cells one by one according to a predefine pattern with good cell survival. This method has been first developed with nanosecond lasers focused on an absorbing layer, the deformation of which can induce the projection of the “ink” from its surface. It is possible to perform LIFT by directly focusing the laser in the volume of the “ink” to be printed. This ink can contain cells, proteins or any biological material of any viscosity. We will present our latest results of 3D bioprinting by laser and the evidence of in-vivo bone repair on mouse critical-size bone calvaria defect thanks to these techniques. Future achievements with the automated 3D laser-assisted bioprinting device will be presented.

8947-65, Session 14

Image-inspired fabrication of 3D biomimetic models of the extracellular matrix via multiphoton-excited photochemistry

Paul J. Campagnola, Visar Ajeti, Ping-Jung Su, Quyen Tran, Jayne Squirrell, Brenda Ogle, Univ. of Wisconsin-Madison (United States)

Biomimetic models of the extracellular matrix afford better investigations of the underlying cell-matrix interactions in normal and diseased states and also provide insight into optimizing scaffolds for tissue regeneration/repair. The optimal fabrication scheme needs to reproduce the native nano/microstructured topography from the same protein components that comprise the native structure. Our fabrication approach uses multiphoton excited (MPE) photochemistry, where analogous to multiphoton fluorescence excitation microscopy, the fabrication is confined to the focal volume, resulting in intrinsic freeform 3D capabilities. Here, proteins are solubilized in an aqueous environment in the presence of a photoactivator and crosslinked, where the resulting feature sizes are governed by the MPE point spread function. Our scaffold designs are derived directly from “blueprints” based on high resolution microscope imaging data (e.g. fluorescence and Second Harmonic Generation (SHG). We introduce a new form of instrument control, modulated raster scanning, that combines aspects of raster and vector scanning control that not only reproduces the features with the same resolution as in the original image data but also where the gray scale intensity is mapped directly the final protein concentration in the fabricated construct. We already have demonstrated the feasibility of this approach by fabricating models of the developing heart (based on Col IV immunofluorescence), the ovarian stroma (based on SHG) and developing mouse heart (based on FN immunofluorescence). We achieved fidelity in terms of feature reproduction and concentration of over 95% relative to the original image. The results are compared to a CAD based STL approach we previously reported.

Conference 8948: Multiphoton Microscopy in the Biomedical Sciences XIV

BiOS SPIE Photonics West

Sunday - Tuesday 2 –4 February 2014

Part of Proceedings of SPIE Vol. 8948 Multiphoton Microscopy in the Biomedical Sciences XIV

8948-1, Session Key

Adaptive optics from microscopy to nanoscopy *(Keynote Presentation)*

Martin Booth, Univ. of Oxford (United Kingdom)

High resolution microscopy relies on the use of high quality optics with the goal of obtaining diffraction-limited operation. Yet in many cases this goal is not achieved as aberrations, distortions in the optical wavefront introduced by the specimen, blur the focus and reduce the resolution of the system. Superresolution microscopes have been able to resolve features on the scale of tens of nanometers and lower. These microscopes - including scanning methods (STED, RESOLFT, etc.) and single molecule switching (SMS) methods (PALM, STORM, GSDIM, etc.) - all suffer from the effects of aberrations that compromise resolution, signal and consequently image quality. Adaptive optics has been demonstrated in a range of diffraction-limited resolution microscope modalities to compensate for system and specimen-induced aberrations. However, the use of adaptive optics in superresolution microscopes presents new challenges. We investigate how aberrations affect the properties of superresolution microscopes and develop new adaptive optics schemes to measure and correct the aberrations. In particular we show aberration correction in 2D and 3D STED microscopes via sensorless image-based feedback schemes. We also show improvement in localization performance in SMS microscopes. The adaptive nanoscopes are used to perform three-dimensionally resolved superresolution imaging through thick (~10 to 50 micrometre) specimens. Significant improvements in resolution or localization precision and image intensity are achieved. The adaptive correction of specimen-induced aberrations in this manner will extend the application of superresolution nanoscopes to thicker specimens.

8948-2, Session Key

In vivo deep tissue multiphoton imaging *(Keynote Presentation)*

Chris Xu, Cornell Univ. (United States)

Multiphoton microscopy has been applied to imaging deep in scattering tissue because of its intrinsic 3D localized excitation. However, the imaging depth of conventional MPM was still limited to less than 1 mm. In this talk, the fundamental challenges of deep tissue, high-resolution optical imaging are discussed. New technologies for deep [1-3] and fast [4] in vivo multiphoton imaging will be presented.

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8948-3, Session Key

Quantitative multiphoton imaging *(Keynote Presentation)*

Karsten König, Aisada Uchugonova, Hans Georg Breunig, Madlen Kloetzer, Univ. des Saarlandes (Germany); Martin Weinigel, Rainer Bückle, JenLab GmbH (Germany); Daria Gaidar, Univ. des Saarlandes (Germany); Jürgen M. Lademann, Charité Universitätsmedizin Berlin (Germany)

The novel certified medical tomograph MPTflex-CARS with its flexible optomechanical arm and compact 360° scan/detection head provides non-invasive and label-free optical skin biopsies with chemical information. Two near infrared laser beams are transmitted through an optical arm and spatially and temporally overlapped within the tissue to generate autofluorescence, SHG, and CARS signals. Lipids, water, and chemotherapeutics have been imaged as well as the fluorescence of elastin, collagen, melanin, NAD(P)H, and flavins with subcelluar resolution. The signals are detected by single photon counting, in part by time-correlated single photon counting (TCSPC). This method provides counts per pixel and opens the way to quantitative imaging. Intracellular concentrations can be determined based on calibration measurements on solutions, cell monolayers, phantoms, biopsies, and clincial data.

8948-4, Session 1

Differentiation of Col I and Col III isoforms in stromal models of ovarian cancer by analysis of second-harmonic generation polarization and emission directionality *(Invited Paper)*

Paul J. Campagnola, Karissa B. Tilbury, Chi-Hsiang Lien, Univ. of Wisconsin-Madison (United States); Shean-Jen Chen, National Cheng Kung Univ. (Taiwan)

A profound remodeling of the extracellular matrix occurs in many epithelial cancers, where for example, the minor collagen isoform of Col III become up-regulated in ovarian cancer. Here we use Second Harmonic Generation (SHG) imaging microscopy to probe differences in structure in fibrillar models of the ovarian stroma comprised of mixtures of Col I and III. The SHG intensity and forward-backward ratios, decrease with increasing Col III content, consistent with decreased phasematching due to more randomized structures. We also probe the net collagen ?-helix pitch angle within the gel mixtures using a new pixel based polarization resolved approach by combining previous analyses. The extracted pitch angles are consistent with those of peptide models and the method has sufficient sensitivity to differentiate Col I from the Col I/ Col III mixtures. The pixel-based approach is also used to determine the SHG signal polarization anisotropy and we found that increased Col III results in decreased alignment of the dipole moments within the focal volume. Lastly, the mixed gels show decreased SHG circular dichroism (CD), indicating the overall chirality is decreased upon Col III incorporation. Collectively, the measurements and analysis all indicate that incorporation of Col III results in decreased organization across several levels of collagen assembly. Further the findings suggest that the collagen isoforms co-mingle within the same fibrils, in good agreement with ultratructural data. The pixel-based polarization analyses (both excitation and emission) afford determination of structural properties without the previous requirement of having well-aligned fibers, and should be generally applicable in tissue.

8948-5, Session 1

Motion-free polarization second-harmonic generation microscopy using a liquid crystal modulator

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Second Harmonic Generation (SHG) microscopy coupled with polarization analysis has great potential for use in tissue characterization, as molecular and supramolecular structural details can be extracted. Such measurements are difficult to perform quickly and accurately, since polarization distortion from the non-45° reflections as well as birefringence and strain in dichroics and other optics on the traversing optical path. Here we present a new method that uses a liquid crystal modulator (LCM) located in the infinity space of a SHG laser scanning microscope that allows the generation of any desired linear or circular polarization state. As the device contains no moving parts, polarization can be rotated accurately and faster than by manual or motorized control. The performance in terms of polarization purity was validated using Stokes vector polarimetry, and found to have minimal residual polarization ellipticity. We also benchmarked the performance by imaging cylindrically symmetric giant unilamellar vesicles (GUV) with stained dye (Di-8-ANEPPS) and the experimental data is well fit to the theoretical prediction. Lastly, the SHG intensity dependence on the linear polarization angle is measured for the well-characterized cases of tendon and skeletal muscle and the extracted helical pitch angles agree with previous results. We also demonstrate the approach for performing accurate SHG circular dichroism (CD) measurements. The LCM has a small footprint and can be implemented easily in any standard microscope and is cost effective relative to other technologies. Thus this approach provides a means to implement polarization-resolved SHG microscopy for a range of applications.

8948-6, Session 1

Non-linear imaging and characterization of atherosclerotic arterial tissue using combined two photon fluorescence, second-harmonic generation and CARS microscopy

Riccardo Cicchi, Istituto Nazionale di Ottica (Italy); Christian Matthäus, Tobias Meyer, Annika Lattermann, Benjamin Dietzek, Institut für Photonische Technologien e.V. (Germany); Bernhard R. Brehm, Catholic Clinic, Koblenz (Germany); Jürgen Popp, Institut für Photonische Technologien e.V. (Germany); Francesco Saverio Pavone, European Lab. for Non-linear Spectroscopy (Italy)

Atherosclerosis is among the most widespread cardiovascular diseases and one of the leading cause of death in the Western World. Characterization of arterial tissue in atherosclerotic condition is extremely interesting from the diagnostic point of view. Routinely used diagnostic methods, such as histopathological examination, are limited to morphological analysis of the examined tissues, whereas an exhaustive characterization requires a morpho-functional approach. Multimodal non-linear microscopy has the potential to bridge this gap by providing morpho-functional information on the examined tissues in a label-free way. Here we employed multiple non-linear microscopy techniques, including CARS, TPF, and SHG to provide intrinsic optical contrast from various tissue components in both arterial wall and atherosclerotic plaques. CARS and TPF microscopy were used to respectively image lipid depositions within plaques and elastin in the arterial wall. Cholesterol deposition in the lumen and collagen in the arterial wall were selectively imaged by SHG microscopy and distinguished by forward-backward SHG ratio. Image pattern analysis allowed characterizing collagen organization in different tissue regions. Different values of fiber mean size, distribution and anisotropy are calculated for lumen and media prospectively allowing for automated classification of atherosclerotic lesions. The presented method represents a promising diagnostic tool for evaluating atherosclerotic tissue and has the potential to find a stable place in clinical setting as well as to be applied in vivo in the near future.

8948-7, Session 1

Second-harmonic generation reveals a relationship between metastatic potential and collagen fiber structure

Kathleen A. Burke, Univ. of Rochester (United States); Ryan Dawes, Univ. of Rochester Medical Ctr. (United States); Mehar Cheema, Stony Brook Univ. (United States); Seth Perry, Edward Brown, Univ. of Rochester Medical Ctr. (United States)

Second Harmonic Generation (SHG) imaging of collagen allows for the analysis of collagen structural changes throughout metastatic progression. The directionality of coherent SHG signals, measured as the ratio of the forward-propagating to backward propagating SHG signal (F/B ratio), is affected by fibril diameter, spacing, and order versus disorder of fibril packing within a fiber. As tumors interact with their microenvironment and metastasize, it causes changes in these parameters, and concurrent changes in the F/B ratio. Specifically, the F/B ratio of breast tumors that are highly metastatic to the lymph nodes is significantly higher than the F/B ratio of tumors with restricted lymph node involvement. We utilized in vitro analysis of tumor cell motility through collagen gels of different microstructures, and hence different F/B ratios, to explore the relationship between collagen microstructures and metastatic capabilities of the tumor. By manipulating environmental factors of fibrillogenesis and biochemical factors of fiber composition we created methods of varying the average F/B ratio of the gel, with significant changes in fiber structure occurring as a result of alterations in incubation temperature and increasing concentrations of type III collagen. A migration assay was performed using simultaneous SHG and fluorescent imaging to measure average penetration depth of human tumor cells into the gels of significantly different F/B ratios, with preliminary data demonstrating that cells penetrate deeper into gels of higher F/B ratio caused by lower type III collagen concentration. Determining the role of collagen structure in tumor cell motility will aid in predicting metastatic capabilities of primary tumors.

8948-8, Session 1

The study of radiation-induced damage and remodeling of extracellular matrix of rectum and bladder by second-harmonic generation microscopy

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Adverse events in normal tissues after irradiation of malignant tumors

are of great importance in modern radiation oncology. There are but a few investigations concerned with the radiation-induced alterations of collagen – the key protein component of the connective tissue matrix. Second harmonic generation (SHG) microscopy allows observe the structure of collagen fibers and bundles without staining. The study objective was evaluation the dose-time dependences of the structural changes occurring in collagen of rat rectum and bladder after gamma-irradiation. Animals were irradiated by a local field at single doses of 2 Gy, 10 Gy and 40 Gy. The study of collagen state was carried out in a day, a week and a month after radiation exposure. Paraffin-embedded material was sectioned on the slices 10 mkm thick and the slices were deparaffinized. SHG-imaging was performed by LSM 510 Meta (Carl Zeiss, Germany). Excitation was implemented with a pulsed (100-fs) titanium-sapphire laser (MaiTai HP, Spectra Physics, USA) at a wavelength of 800 nm and a pulse repetition frequency of 80 MHz. In a day after irradiation, sings of epithelial damage and oedema of submucosal layer, more significant after the dose of 40 Gy were observed on LSM-images. In a week after irradiation increase of a number and a size of collagen fibers was revealed independently on the dose. In a month the fibrosis appearances became more significant. SHG microscopy gives a valuable information concerning the processes of radiation-induced changes of normal tissues in addition to standard and special histological staining.

8948-9, Session 1

Towards a compact fiber laser for multimodal imaging

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Due to benefits such as high contrast ratio, submicron resolution and depth resolved imaging, multiphoton microscopy has gained broad acceptance in recent years. Signal is known to increase as the inverse of pulse duration for SHG and the inverse of the pulse duration squared for THG, without bandwidth limits imposed by the two-photon absorption spectrum; making SHG and THG amenable to ultrashort pulse excitation. Laser pulse duration dependence has been confirmed for two-photon microscopy down to 10 fs pulses. In the past decade, compact fiber lasers have drawn increasing attention due to their compact size and greater stability. Er fiber at 1550 nm and Yb fiber at 1060 nm, have been used for multiphoton microscopy; however, these lasers have pulse durations greater than 100 fs. In order to avoid laser induced damage to living tissue and enable microscopic imaging deeper into living tissue, interest has shifted towards imaging using longer wavelength sources. Here present a new fiber laser source delivering a very broadband spectrum centered at 1030 nm. The output pulses can be de-chirped to as short as ~21 fs using a pulse shaper with Multiphoton Intrapulse Interference Phase Scan (MIIPS). To the best of our knowledge, this is the shortest pulse duration obtained directly from a fiber oscillator. This source is evaluated for multi-modal microscopy using fluorescent polystyrene microspheres and unstained biological samples including guppy fish (Poecilia reticulata) tails and fruit fly (Drosophila melanogaster) wings. Images generated by multiphoton fluorescence, SHG and THG are compared.

8948-11, Session 2

Nonlinear imaging of collagen cross-links in developing tendon

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Collagen cross-links afford mechanical strength to tendon tissues and are critical in guiding tendon development and repair. Assessing changes in collagen cross-linking has implications for tendon tissue engineering as well as for understanding collagen structure with respect to musculoskeletal tissue development. Here, we applied two-photon excited fluorescence (TPEF) and second-harmonic generation (SHG) microscopy to probe collagen cross-links in tendon tissues excised from chicken embryos at different development stages. Endogenous TPEF from hydroxylysyl pyridinoline (HP) and lysyl pyridinoline (LP) trivalent cross-links was observed in the epi-direction at 400 nm (± 20 nm) by optical excitation at 720 nm from a mode-locked Ti:sapphire laser through a 63x (1.2 NA, water-immersion) objective lens. Forward- and backward- emitted SHG from collagen fibrils was collected using an 800 nm fundamental wavelength. Differences in optical properties associated with tissue thickness were minimized by longitudinally cryosectioning specimens to 50 μm. A reduced cross-linking tendon model were also examined by treating embryos in ovo with ?-aminopropionitrile (BAPN) to inhibit lysyl oxidase, an enzyme that mediates collagen cross-link formation. Optical measurement s were compared with quantification of HP- and LP-density and collagen content in analogous tendons specimens via tandem mass spectrometry (LC-MS/MS). Increases in collagen content with developmental age as measured by SHG were consistent with LC-MS/MS results. Meanwhile, both TPEF and LC-MS/MS methods also identified variations in HP and LP cross-link density between development stages and significant reductions in cross-link density with BAPN treatment.

8948-12, Session 2

Photonic structure of chitin-protein organization in squid internal shell observed by second-harmonic generation (SHG) and electron microscopy

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Chitin is a linear homopolysaccharide composed of N-acetylglucosamine chains. It is found in arthropod exoskeleton, mollusk shells and fungal cell wall. We have performed a systematic analysis of the chitin-protein complexes and organization in the internalized and non-calcified shell in squids. Although not developed as a calcified shell, the internal shell or pen, performs and important function in body structure and resistance. The multiple birefringent layers of the squid pen act as a photonic structure. We identified the layered structure using transmission electron microscopy and observed a series of fracture planes, which, by close examination and morphometric analysis, unveiled an angular variation between the repeating in-plane fracture lines associated with the basic chitin microfibril protein complexes. Transmission electron microscopy allowed the confirmation of repeating optical units with preferential alignment and organization, fitting well with published repeating units revealed by X-ray diffraction studies. Under femtosecond pulsed laser imaging we could detect transmitted and reflected SHG, which were mutually exclusive, creating a interference pattern resembling cholesteric crystals. On further inspection of the 3D structure, the transmitted and reflected SHG were shown to originate from individual and intercalating layers. This phenomenon was interpreted as the result of the very organized pattern of the photonic birefringent structure on the light produced by SHG. We will discuss the implications of the present findings to other structures exhibiting SHG such as collagen fibers.

8948-13, Session 2

Tunable pulse compensation for significant improvement of signal in nonlinear endomicroscopy imaging

Gunnsteinn Hall, Wenxuan Liang, Johns Hopkins Univ. (United States); Ming-Jun Li, Corning Inc. (United States); Zaver Bhujwalla, Kristine Glunde, Johns Hopkins Univ. (United States); Katherine Luby-Phelps, Mala Mahendroo, The Univ. of Texas Southwestern Medical Ctr. at Dallas (United States); Xingde Li, Johns Hopkins Univ. (United States)

Scanning fiber-optic, nonlinear optical endomicroscopy is an emerging technology that can potentially translate high-resolution multiphoton imaging to in vivo clinical applications. It is critical to maintain a short temporal pulse width in a fiber-optic endomicroscope for achieving efficient nonlinear signal production without the need for increasing the average excitation laser power. Adopting the double fiber scheme with anomalous dispersion in the middle, initially developed by Clark et al., we have reduced the pulse width at the endomicroscope output from 400 fs down to ~60-70 fs, increasing the imaging signal by approximately four-fold. We quantify the improvements through two-photon imaging of fluorescein at increasing power levels, as well as through frequency-resolved optical gating (FROG) that is used for pulse characterization. We also performed second-harmonic generation (SHG) and two-photon fluorescence endomicroscopic imaging of biological tissues, demonstrating high-quality images with much improved signal-to-noise ratio (SNR). In addition, for the first time to our knowledge, we show that the method is feasible at both 780 and 890 nm, making the technique easily tunable and applicable to a range of imaging needs. The pulse width is eventually limited by residual third-order dispersion (TOD), particularly at 890 nm where the TOD of fused silica is higher. However, in contrast to some previous experiments, FROG analysis of our pulses at typical incident powers demonstrates that TOD is not a significant limitation and our pulses remain near-bandwidth limited. A more detailed analysis of this will be presented.

8948-14, Session 2

Label-free multiphoton imaging using a compact femtosecond fiber laser mode-locked by carbon nanotube saturable absorber

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We demonstrate label-free multi-photon imaging of biological samples using a compact Er3+-doped femtosecond fiber laser mode-locked by a single-walled carbon nanotube (CNT). These compact and low cost lasers have been developed by various groups but they have not been exploited for multiphoton microscopy. Here, it is shown that various multiphoton imaging modalities (e.g. second harmonic generation (SHG), third harmonic generation (THG), two-photon excitation fluorescence (TPEF), and three-photon excitation fluorescence (3PEF)) can be effectively performed on various biological samples using a compact handheld CNT mode-locked femtosecond fiber laser operating in the telecommunication window near 1560nm. We also show for the first time that chlorophyll fluorescence in plant leaves and diatoms can be observed using 1560nm laser excitation via three-photon absorption.

8948-15, Session 2

Fiber array-based time-multiplexed multifocal multiphoton microscopy (TM-MMM): a new approach toward large numbers of time multiplexing

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TM-MMM, a multifocal imaging technique that increases its parallelization by introducing distinct temporal delays (DTDs), has created the possibility of achieving superior optical sectioning and imaging speeds at the same time. Theoretically, the optimized (i.e., scanningless) TM-MMM requires a temporal mask, conventionally made from an array of glass cylinders with various heights, to generate a large number of DTDs (~300); however, to avoid the light leakage between adjacent temporal channels caused by free-space diffraction, the physical dimensions of such a temporal mask are physically impractical. In this paper, we demonstrate a novel design of the temporal mask that can create more than 5 times of the DTDs provided by conventional masks (~3). This novel temporal mask consists of an array of single-mode optical fibers of various lengths. The single-mode operation of the fibers confines the propagating mode from spreading over a practically arbitrary length, and therefore the length differences in the temporal channels are no longer limited by free-space diffraction as in the case of conventional masks. Currently the main limiting factor of our technique is the precision of fiber length control, and we expect additional 10-fold increase of the number of DTDs upon improving the precision from 100 ?m to 10 ?m. The performance of our proposed fiber array-based temporal mask is demonstrated through the results of numerical simulations, measurement of axial response, and imaging of biological specimens.

8948-81, Session 2

In vivo time-lapse imaging of skin burn wound healing using second-harmonic generation microscopy

Takeshi Yasui, Univ. of Tokushima (Japan); Ryosuke Tanaka, Osaka Univ. (Japan); Eiji Hase, Univ. of Tokushima (Japan); Shu-ichiro Fukushima, Tsutomu Araki, Osaka Univ. (Japan)

Wound healing is a process to repair the damaged tissue. Although wound healing has many aspects, it is common for dynamics of collagen fiber, such as decomposition, production, or growth, to be closely related with wound healing. If such healing process can be visualized from the viewpoint of the collagen dynamics, one may obtain new findings regarding biological repairing mechanisms in the healing process.

Second-harmonic-generation (SHG) light functions as an effective optical probe that shows high selectivity and good image contrast to collagen molecules as well as high spatial resolution, optical three-dimensional (3D) sectioning, minimal invasiveness, deep penetration, the absence of interference from background light, and in vivo measurement without additional staining. Furthermore, since SHG light arises from a non-centrosymmetric triple helix of three polypeptide chains in the collagen molecule, its intensity decreases and finally disappears when thermal denaturation caused by the skin burn changes the structure of this molecule to a centrosymmetric random coil. Therefore, optical assessment of skin burn has been investigated by SHG microscopy. In this paper, we applied SHG microscopy for in vivo imaging of the healing process in animal skin burn and successfully visualized the decomposition, production, and growth of dermal collagen fibers as a series of time-lapse images in the same subject.

8948-16, Session 3

Exploring the brain on multiple scales with correlative two-photon and light sheet microscopy *(Invited Paper)*

Ludovico Silvestri, Anna Letizia Allegra Mascaro, Irene Costantini, Univ. degli Studi di Firenze (Italy); Leonardo Sacconi, National Institute of Optics (Italy); Francesco Saverio Pavone, Univ. degli Studi di Firenze (Italy)

One of the unique features of the brain is that its activity cannot be framed in a single spatio-temporal scale, but rather spans many orders of magnitude both in space and time. A single imaging technique can reveal only a small part of this complex machinery. To obtain a more comprehensive view of brain functionality, complementary approaches should be combined into a correlative framework. Here, we describe a method to integrate data from in vivo two-photon fluorescence imaging and ex vivo light sheet microscopy, taking advantage of blood vessels as reference chart [1]. We show how the apical dendritic arbor of a single cortical pyramidal neuron imaged in living thy1-GFP-M mice can be found in the large-scale brain reconstruction obtained with light sheet microscopy. Starting from the apical portion, the whole pyramidal neuron can then be segmented. The correlative approach presented here allows contextualizing within a three-dimensional anatomic framework the neurons whose dynamics have been observed with high detail in vivo.

This work has received funding from LASERLAB-EUROPE (grant agreements n° 228334 and 284464, EC's Seventh Framework Programme) and has been supported by the Italian Ministry for Education, University and Research in the framework of the Flagship Project NANOMAX, by Italian Ministry of Health in the framework of the 'Stem Cells Call for proposals'.

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1. L. Silvestri et al. Correlative two-photon and light sheet microscopy, in press

8948-17, Session 3

Fiber-optic scanning nonlinear endomicroscopy *(Invited Paper)*

Xingde Li, Wenxuan Liang, Gunnsteinn Hall, Jiefeng Xi, Johns Hopkins Univ. (United States); Ming-Jun Li, Corning Inc. (United States); Zaver Bhujwalla, Kristine Glunde, Johns Hopkins Univ. (United States); Katherine Luby-Phelps, Mala Mahendroo, The Univ. of Texas Southwestern Medical Ctr. (United States)

Nonlinear microscopy, such as two-photon fluorescence (TPF) and second harmonic generation (SHG) microscopy, has had significant impact on basic and applied research. The scope of in vivo clinical applications of this technology, however, remains very limited due to the inaccessibility of the bulky microscope-based imaging platform to many organs (except skin). Recent years have witnessed increasing interest and rapid advances in developing miniature TPF/SHG endomicroscopes. This talk will briefly review the development of the fiber-optically based, scanning nonlinear endomicroscopy technology. Since the first all-fiber-optic scanning TPF endomicroscope reported by our group in 2006, many technical and engineering challenges have been identified and overcome. The scanning head is one of the key components in the nonlinear endomicroscopy technology, and in our design, it consists of 1) a specially designed double-clad fiber for delivery of femtosecond excitation light and effective collection of nonlinear signal, 2) a compact and fast 2D beam scanner, and 3) a micro objective lens. High-quality submicron-resolution nonlinear endomicroscopy imaging of biological tissues is possible without the need for staining. Some representative TPF and SHG images related to tissue viability (during transplantation), breast cancer detection, and preterm birth risk assessment etc. will be presented. The results suggest the potential of visualizing tissue histology in situ, in vivo and in real time with the nonlinear endomicroscopy technology based on intrinsic tissue metabolic (such as NADH and FAD) and structural (such as collagen) biomarkers. Other potential applications such as brain function imaging will also be discussed.

8948-18, Session 3

Advances in laser sources for nonlinear imaging

Darryl McCoy, Coherent Scotland Ltd. (United Kingdom); Marco F. Arrigoni, Coherent, Inc. (United States)

The demand in the biological sciences for new and novel laser sources for Multiphoton Excitation microscopy is continuous. The challenge for a commercial laser company is that by nature, this demand is often dispersive - with differing requirements in terms of laser power, repetition rate, and pulse width. On the other hand, there are many convergent trends, in terms of target fluorescent probes, and the drive for longer and multiple wavelengths.

The relationship between average power, pulse width and peak power from a laser system and the corresponding effect to image quality, intensity and indeed to the biological sample itself remains a polarizing topic for many biologists and technologists in non-linear imaging. We explore in this presentation the considerations that can be made in selection and configuration of laser system with applications examples. Key attention is given to the relationship between sample damage and peak laser intensity.

Within this backdrop we show latest advances in Coherent lasers for non-linear imaging. In particular how they address the confluence of wavelength demands for applications in neuroscience and developmental biology.

With much on-going work in the field on red fluorescent probes and voltage sensitive dyes, together with three photon and third harmonic generation studies, we also outline how the latest Coherent technologies deliver reliable and flexible long wavelength capability.

8948-19, Session 3

Latest advances in ultrafast laser sources for multiphoton microscopy

Philip G. Smith, Spectra-Physics, a Newport Corp. Brand (United States)

The advent of compact, fully automated, and widely wavelength-tunable ultrafast oscillators has triggered an explosive growth in their use in a broad array of multiphoton imaging techniques. Over the past decade laser manufacturers have constantly improved the performance characteristics of these sources to meet the requirements of the user community. We will review the latest advances at Newport / Spectra-Physics in this field and discuss new ways of optimizing key parameters for efficient deep-tissue fluorescence generation, including turn-key, automated second order dispersion compensation that allows for optimization of the pulse width at the sample over a wide wavelength range, without compromising beam pointing and other critical beam parameters.

8948-20, Session 3

3D-resolved optical targeting for photodynamic therapy

Christopher J. Rowlands, Jackie Wu, Peter T. C. So, Massachusetts Institute of Technology (United States)

We present recent developments in our attempts to develop optically-targeted photodynamic therapy. In conventional photodynamic therapy, selectivity in targeting is mostly chemical in origin; the photodynamic agent is more toxic to cancer cells than the surrounding tissue, for the same incident light intensity. In therapeutic use, control over the incident photon flux, which offers much greater selectivity and greater precision than chemoselectivity, is currently limited to methods that confine the light to a broad region surrounding the tumor. We aim to combine temporal focusing (an optical technique that permits widefield two-photon patterning with good depth resolution) with photodynamic therapy in order to more precisely target the cells of interest.

In this presentation, we will discuss the design of the temporal focusing instrument, illustrating how various design parameters were met. In particular, we will discuss how to achieve a large field-of-view while simultaneously maintaining good axial sectioning in tissue. We will also discuss how our method is applicable to a wide range of photosensitizers; since targeting is performed by controlling the incident photon flux, photosensitizers do not need to be optimized for specificity towards cancer cells, and can instead be optimized for greater absorption cross-section or singlet-oxygen yield.

Finally, we will demonstrate that it is possible to selectively kill cancer cells using this approach; we will show that many OVCAR5 ovarian cancer cells can be simultaneously targeted with 3D resolution, and that apoptosis is limited to the exposed regions.

8948-21, Session 3

Improving the optical sectioning capability of temporally focused widefield two-photon microscopy

Elijah Y. S. Yew, Singapore-MIT Alliance (Singapore); Peter T. C. So, Massachusetts Institute of Technology (United States)

Temporally focused widefield two-photon microscopy (TF2P) has shown to be useful in several applications ranging from high-throughput FLIM/PLIM, spatially modulated excitation of neurons (optogenetics), tissue ablation, and microfabrication. The drawback to TF2P is that the axial extent over which two-photon absorption occurs may be relatively wide (FWHM approximately 9 um) as compared to a diffraction limited spot. This is because the spectrally dispersed pulse is often focused to a line and subsequently does not fully utilize at the back focal plane of the objective. While some research have illustrated the ability of reduce this to around 0.8 um, this often requires aligning two gratings to the back focal plane of the objective. In this presentation, we present an alternative method of improving the axial extent of a TF2P system. We find that by over-filling the back focal plane of the objective and projecting a standing wave pattern to the sample reduces the axial FWHM to 2 um. We propose that this method has advantages in areas of imaging as well as fabrication because it is based on a simple design and the axial resolution can be varied simply by changing the period of the standing wave pattern.

8948-71, Session PSun

Multiphoton microscopy for skin wound healing study in terms of cellular metabolism and collagen regeneration

Gitanjal Deka, National Yang-Ming Univ. (Taiwan); Kazunori Okano, National Chiao Tung Univ. (Taiwan); Wei-Wen Wu, National Yang-Ming Univ. (Taiwan) and Taipei City Hospital (Taiwan); Fu-Jen Kao, National Yang-Ming Univ. (Taiwan)

Multiphoton microscopy is employed to study normal skin wound healing in live rats noninvasively. Wound healing is a process involving series of biochemical events. This study evaluates the regeneration of collagen and change in cellular metabolic activity during wound healing in rats, with second harmonic generation (SHG) and fluorescence lifetime imaging microscopy (FLIM), respectively. In eukaryotic cells ATP is the molecule that holds the energy for its functioning. Whereas NADH is an electron donor in the metabolic pathways, required to generate ATP. Fluorescence lifetime of NADH free to protein bound ratio has been evaluated to determine the relative metabolic activity. The FLIM data acquisition were done by a TCSPC system using SPCM software and analyzed by SPCImage software. Additionally, polarization resolved SHG signals were also collected to observe the changes in birefringence of regenerated collagens from rat wound biopsy samples. Mat lab programming is used to process the data to acquire the anisotropy images. Results indicate that cells involved in healing have higher metabolic activity during the first week of healing, which decreases gradually and become equivalent to normal skin. A net degradation of collagen during the inflammatory phase and net regeneration starting from day 5 were observed in terms of SHG signal intensity change. Polarization resolved SHG imaging of the wound biopsy sample indicates higher birefringence in first week of the healing; however the birefringence decreases upon healing.

8948-72, Session PSun

Influence of photon bunching on two-photon excited fluorescence

Henning Kurzke, Andreas Jechow, Michael Seefeldt, Axel Heuer, Ralf Menzel, Univ. Potsdam (Germany)

Two-photon excited fluorescence (TPEF) has become a standard technique in modern microscopy [1]. In this work we report on utilizing the photon bunching effect in thermal light to enhance the efficiency of TPEF under continuous wave illumination. We used a super-luminescence diode (SLD) as thermal light source and a DFB diode laser emitting at the same wavelength as coherent light source. The degree of second order coherence g(2) of both sources was measured following a scheme described in [2]. The SLD showed photon bunching with g(2) = 1.90±0.2 as expected for thermal light, while the DFB diode laser emitted coherent light exhibiting no photon bunching at g(2) = 1.

TPEF signals from common fluorophores and water soluble luminescent quantum dots were obtained with both light sources. By comparing the ratio of the TPEF signals of thermal and coherent excitation we obtain an enhancement of a factor of almost two for the tested dyes and quantum dots, respectively.

Our results show that the quantum nature of thermal light can be exploited in TPEF experiments with the photon statistic providing a new degree of freedom. This has potential application in two-photon microscopy. Since also higher order photon bunching was observed with thermal light [3], this scheme can be extended to multi-photon absorption processes with N photons resulting in higher enhancement factors of N!.

[1] W. Denk et. al, Science 248, 73-76, (1990);

[2] F. Boitier et. al, Nat. Phys. 5, 267-270 (2009);

[3] M. Assmann et. al, Science 325, 297-300 (2009);

8948-73, Session PSun

In pixel analysis of molecular structure with Stokes vector resolved second harmonic generation microscopy

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We report on measurement and characterization of polarization properties of Second Harmonic (SH) light using a four-channel photon counting based Stokes-polarimeter. Various polarization parameters, such as the

degree of polarization (DOP), the degree of linear polarization (DOLP), the degree of circular polarization (DOCP) are extracted by implementing a pixel by pixel image analysis from the 2D reconstructed SH Stokes images of SHG active samples. Although, the Stokes parameters can measure and analyze the polarization states of SH light, but are not able to examine the perfect alignment and orientation of molecules. Therefore, we extended the measurement by varying the polarization state of the incident light and detecting different polarization components of the SH signal via our PSA.

8948-74, Session PSun

Shedding light into atherosclerosis: a quantitative study of nonlinear optical imaging in tracking plaque development

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Atherosclerosis is a vascular disease which is commonly characterized by an arterial plaque built up, limiting the blood flow to vital organs and thus causing life-threatening conditions such as heart attack or stroke. Despite recent progress in clinical imaging, early atherosclerosis still cannot be reliably detected. In recent years, nonlinear optical imaging microscopy (NLOM) has proven itself as a powerful tool for high-resolution, label-free visualization of extracellular bio-morphology in the plaques. While most studies presented valuable insight into the composition and structural fingerprints of atherosclerotic lesions, very few works actually studied the correlation between such extracellular image features with disease progression. In this study, we focus on establishing a numerical index that can be easily used to track atherosclerotic plaque development based on NLOM image statistics. With imaging data collected from 24 WHHL rabbits this index demonstrated a strong correlation with the severity of the atherosclerotic lesions, represented by the rabbits' age. Using this index we could also identify several high-risk locations for plaque formation along the blood vessel. This result was validated with immunohistochemical fluorescence imaging.

8948-75, Session PSun

Two-photon in vivo imaging of retinal microstructures

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Non-invasive fluorescence fundus imaging could prove to be an important tool for in-vivo small animal retinal imaging in a wide array of translational vision applications, including the tracking of fluorescently tagged cells and the expression of gene-therapy and optogenetic vectors. Recently, we demonstrated the ability to obtain cellular resolved images in a retina transduced with fluorescent proteins and the GCaMP-family of optogenetic calcium indicators using fluorescence micro-endoscopy (Schejter et al,. 2012, TVST). Obtaining these capabilities with two-photon microscopy could have the added advantages of optical sectioning, reduced photodamage and photobleaching, and of being based on infrared light.

Here, we demonstrate that two-photon laser scanning microscopy through a mouse's pupil can yield high-quality fundus images of fluorescent retinal micro-structures in-vivo. Two-photon in-vivo microscopy was performed through the dilated pupil in head-fixed animals with a 10x water-immersion objective. These imaging sessions yielded well-resolved fluorescein angiograms and images of optogenetic probes' expression which were axially sectioned to individual retinal layers. Interestingly, both two-photon imaging (without adaptive optics) and the endoscope-based images appeared to be robust to PSF distortions that completely smeared out cellular details in a conventional 1P fluorescence microscopic image of the same retina.

These results demonstrate the feasibility of performing two-photon fundus imaging in-vivo, an important step towards various applications such as structural and functional autofluorescence retinal imaging and functional calcium imaging of responses to natural stimuli (using invisible infrared light).

8948-76, Session PSun

Super-nonlinear fluorescence microscopy for high-contrast deep tissue imaging

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Two-photon excited fluorescence microscopy (TPFM) offers the highest penetration depth with subcellular resolution in light microscopy, due to its unique advantage of nonlinear excitation. However, a fundamental imaging-depth limit, accompanied by a poor signal-to-background contrast, still exists for TPFM when imaging scattering samples. Formally, the focusing depth, at which the in-focus signal and the out-of-focus background are equal, is defined as the fundamental imaging-depth limit. To go beyond TPFM, we have recently proposed and demonstrated a new class of super-nonlinear fluorescence microscopy techniques for high-contrast deep tissue imaging (Opt. Exp. 2012; Biomed. Opt. Exp. 2012; J. Phys. Chem. Lett., 2012). Among them, multiphoton activation/deactivation and imaging (MPAI/MPDI) harnesses novel photo-activatable or switchable molecular probes to render the focal volume and the out-of-focus background be occupied by different meta-stable states of fluorescent probes. The resulting image contrast exhibits a fourth-order nonlinear dependence on laser intensity. In addition, in stimulated emission reduced fluorescence (SERF) microscopy, two-photon fluorescence generated at the focus is preferentially switched on and off by a modulated laser beam inducing stimulated emission of the fluorophores from the excited states. The resulting image, constructed from the reduced fluorescence signal, exhibits a 1.8 times depth extension owing to its overall third-order nonlinearity. In all these techniques, the created super-nonlinearity significantly enhances the imaging contrast and concurrently extends the imaging depth-limit. Conceptually different from conventional multiphoton processes mediated by virtual states, our strategy constitutes a new class of fluorescence microscopy where high-order nonlinearity is mediated by real population transfer.

8948-77, Session PSun

Multiphoton microscopy using frequency-doubled compact femtosecond erbium-doped fiber laser

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Multiphoton microscopy (MPM) is a powerful imaging technique but the traditional design requires a Ti: sapphire laser which is bulky, expensive, not portable, and require precise alignment. Currently, there is a trend to replace the Ti:sapphire laser with a more compact, portable, and low cost femtosecond fiber laser as the light source. Although MPM systems using femtosecond fiber lasers at 1.55 μm or 1.0 μm wavelengths have the advantages of deep imaging depth and minimal autofluorescence background, they also have disadvantages such as higher water absorption, lower resolution, and requiring staining of the sample compared to systems using 800 nm wavelength. To fulfill the potential

of MPM systems for in vivo imaging, we developed a compact MPM system based on a frequency-doubled femtosecond erbium-doped fiber laser source at 1.55 µm. By use of periodically poled MgO:LiNbO3, the frequency-doubled pulses at 790 nm with average power of 75 mW and pulse width of 200 fs are applied as the excitation source. The pulsewidth and bandwidth of the fiber laser are optimized to maximize the MPM signal at the sample. A gimbal-less two-axis MEMS scanner is utilized to perform XY scanning for MPM imaging. A miniature objective and multimode fiber are further used to build the compact MPM system. Two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG) images are obtained from unstained biological samples, such as fish scale and mouse tail tendon. In conclusion, the MPM system with a compact, portable, low-cost, frequency-doubled fiber laser has a great potential to transform the current bench-top MPM system to a portable system for in vivo MPM imaging.

8948-78, Session PSun

Comparison of near-infrared confocal and multiphoton microscopy modalities in deep tissue imaging using cyanine contrast agents

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The era of molecular medicine has ushered the development of microscopic methods that can report molecular processes in thick tissues with high spatial resolution. A commonality in deep tissue microscopy is the use of near-infrared (NIR) lasers with single- or multi-photon excitations. However, the relationship between different NIR excitation microscopic techniques and the imaging depths in tissue has not been established. We compared such depth limits for three NIR excitation techniques: NIR single-photon confocal microscopy (NIR SPCM), NIR multiphoton excitation with visible detection (MPM1), and all-NIR multiphoton excitation with NIR detection (MPM2). Homologous cyanine dyes provided the fluorescence. Intact kidneys were harvested after administration of kidney clearing cyanine dyes in mice. NIR SPCM and MPM1 achieved similar maximum imaging depth of ~100 µm. The MPM2 enabled more than five-fold imaging depth (>500 µm) using the harvested kidneys. Although the MPM2 used 1550 nm excitation where water absorption is relatively high, cell viability and histology studies demonstrate that the laser did not induce photothermal damage at the low laser powers used for the kidney imaging. This study guides on the imaging depth capabilities of different microscopy techniques with NIR excitation and reveals the potential of multiplexing information using these systems.

8948-79, Session PSun

Assembly and characterization of a nonlinear optical microscopy for in vivo and ex vivo tissue imaging

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The purpose of this study is assembly and characterization of a custom-made non-linear microscope. The microscope allows the adjustment for in vitro, in vivo and ex vivo imaging of biological samples. Two galvo mirrors conjugated by two spherical mirrors are used for the lateral scan and for the axial scan a piezoeletric stage is utilized. The excitation is done using a tunable femtosecond Ti: Sapphire laser. The light is focused in tissue by an objective lens 20X, water immersion, numerical aperture of 1.0, and working distance of 2.0 mm. The detection system is composed by a cut off filter that eliminates laser light back reflections and diverse dichroic filters can be chosen to split the emitted signal for the two photomultiplier detector. The calibration and resolution of the microscope was done using a stage micrometer with 10 µm divisions and fluorescent particle slide, respectively. Fluorescence and second harmonic generation images were performed using epithelial and hepatic tissue, the images have a sub-cellular spatial resolution. Further characterization and differentiation of tissue layers can be obtained by performing axial scanning. By means of the microscope it is possible to have a three dimensional reconstruction of tissues with sub-cellular resolution.

8948-80, Session PSun

Multiphoton microscopy of ventricular changes in porcine acute and chronic myocardial infarction models

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The change in fiber orientation following myocardial infarction plays a central role in functional adaptation of the heart to ischemic injury. However, understanding of the remodeling process has been limited by the lack of 3D high-resolution visualization. This study aims to determine if bio-photonic techniques can be used to identify myocardial structures for normal, acute and chronic infarct tissues in pig hearts. METHODS: Formalin-preserved, post-mortem cardiac tissues (4 swine) were imaged with a homebuilt 3fps two-photon microscope with ~0.53 x 2.4µm lateral x axial resolution and at 780nm excitation wavelength. Additionally, infarcts were imaged with a SS-OCT system at a central wavelength of 1310nm at ~16.0x9.0µm resolution. Images were post-processed for 3D visualization and fiber angle calculations. RESULTS: In normal porcine heart, perimysial collagen was clearly visualized ~4 cells apart (59µm); reasonable fiber angles at the epicardium, midwall and endocardium were calculated. In sub-acute infarcts (8days post-infarction), necrosis and disintegration of cardiac fibers were observed; fiber tract orientations were largely preserved (9.56o/mm). In chronic infarcts (>100days post-infarction), cardiac remodeling was observed with significant collagen deposition and complete disruption of fiber orientations. Finally, FAD and SHG imaging demonstrated superior 2D visualization of myosin and collagen structures when compared with standard H&E. Our results demonstrated excellent 2D and 3D visualization of cardiac muscle orientation and structures without histological slicing of the heart and without the need of contrast agents. Future investigation of bio-photonic imaging evaluation post-MI remodeling and myocardial disease affect collagen structure including additional targeted histological stains is warranted.

8948-82, Session PSun

Evaluating collagen morphology and pathological lipid deposition using multiphoton image statistics

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A novel image analysis methodology for quantifying and classifying morphological details in tissue collagen organization and lipid deposition is presented. Textural features based on image statistics such as first-order statistics (FOS) and gray level co-occurrence matrix (GLCM) parameters were extracted from the SHG (second harmonic) and CARS (coherent Raman) images of histological sections of myocardial infarcted hearts and atherosclerotic artery walls . The strength of texture analysis in providing quantitative descriptors for multi-photon microscopic images was evaluated by multi-group classifications using support vector machine (SVM) of SHG and CARS images acquired from diseased and healthy tissues samples. Using animal models, different collagen remodelling and lipid accumulation patterns in disease tissues can be successfully tracked using these image statistics thus providing a solid foundation for classification. Using two disease models we have demonstrated the feasibility of performing classification of collagen fibril morphology and lipid accumulation based on first-order and second-order texture statistical parameters derived from SHG and CARS images. Using a nonlinear SMV classifier, it is shown that in more complex cases, the classification accuracy can be improved with combined FOS and GLCM texture variables, compared to the case when either one is used. On the other hand, in a binary classification of myocardial infarcted hearts (treated and non-treated with adipose-derived stem cells) one group of texture parameters was sufficient to generate classification accuracy of better than 90%. The proposed methodology can be used in a wide variety of applications to evaluate conditions involving collagen remodelling and prominent lipid accumulation.

8948-83, Session PSun

Back-reflected SHG detection in corneal histological sections

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Second harmonic generation (SHG) is a coherent process. As so, photons are mainly emitted in the forward direction. Forward SHG detection is desirable, as back-reflected SHG detected signal is small.

In this work, we propose a method to increase SHG signal in the back-reflected direction, reflective backward SHG detection.

Fresh as well as old corneal histological sections (dated back to 2010) were exposed to 12 fs NIR laser pulses to induce SHG and fluorescence. An inverted laser scanning microscope equipped with a 16 channel PMT array with short rise-time was employed to perform two-photon imaging and spectral FLIM. In order to increase the SHG/fluorescence signal, three different reflective materials were placed on top off the sample: aluminum foil, a silver mirror, and a special dichroic mirror with high reflectivity around 400 nm.

The same sample was imaged (within the same area) with and without the reflective materials on top of the sample. Since the weight of the materials causes sample compression, the focal plane had to be readjusted. To ensure that the images were taken at the same sample depth, the correlation was computed. Image pairs with correlation below 0.95 were rejected. The remaining measurement parameters were kept constant. A signal increase of more than 20% was observed when using high NA objectives.

In addition, two-photon excited HE-fluorescence was detected.

This study demonstrated the feasibility to extract novel data such as SHG of collagen of old HE stained histological sections.

8948-84, Session PSun

Visualizing deuterated cholesterol uptake through the LDL endocytic pathway

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Altered cholesterol homeostasis has major impact in numerous physiological processes, which are manifest in a wide range of illnesses from cardiovascular diseases to neurological disorders. Receptor mediated endocytosis of low-density lipoprotein (LDL) particles is an important mechanism by which cells in peripheral tissues receive dietary sterols. In combination with cholesterol biosynthesis, the LDL endocytic pathway is highly regulated to control intracellular cholesterol levels. In this study, we use coherent Raman scattering microscopy to characterize the uptake of deuterated cholesterol by cells through the LDL endocytic pathway. The use of deuterated markers, which were synthesized for this study, increases the chemical specificity of the observations and provides direct evidence of cholesterol imaging. By tuning the Raman shift to the C-D vibrational frequency, d-cholesterol containing particles fed to the cells can be isolated and specifically monitored during the endocytic process. We analyze the intracellular distribution of cholesterol as a function of LDL concentration and incubation time, by quantifying the number of lipid droplets of esterified cholesterol. This study reveals the cellular response to different dietary loading levels and unambiguously shows that d-cholesterol is incorporated into living cells, a process that can be specifically monitored with coherent Raman scattering microscopy. With this strategy we aim to obtain a direct view of cholesterol homeostasis in general and the receptor mediated LDL endocytic pathway in particular.

8948-85, Session PSun

Stepwise multiphoton activation fluorescence reveals a new method of melanoma imaging for dermatologists

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Previous research has shown that the stepwise multi-photon activated fluorescence (SMPAF) of melanin is a low cost and reliable method of detecting melanin because the activation and excitation can be a continuous-wave (CW) mode near infrared (NIR) laser. In this study, SMPAF images of melanin in a mouse melanoma are compared with conventional multi-photon fluorescence microscopy (MPFM) images and confocal reflectance microscopy (CRM) images. All images are acquired at an excitation wavelength of 920 nm to prove the effectiveness of SMPAF in melanin detection. SMPAF images add specificity for melanin detection than MPFM images and CRM images. SMPAF images also demonstrate potential to increase sensitivity for detecting small size melanin granules. Melanin SMPAF is a promising technology to enable early detection of melanoma for dermatologists.

8948-86, Session PSun

Diagnosis of basal cell carcinoma by two photon excited fluorescence combined with lifetime imaging

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Basal cell carcinoma (BCC) is the most common type of human skin cancer in the world. The traditional diagnostic procedure of BCC is histological examination with haematoxylin and eosin staining of the tissue biopsy. Recently, to reduce the complexity of the diagnosis procedure, a number of noninvasive optical methods have been applied in skin examination. As a nonlinear process, multiphoton tomography (MPT) makes use of light in the near-infrared range for multi-photon absorption. Fluorescence lifetime imaging microscopy (FLIM) is one of the most sensitive and quantitative approaches to examine the microenvironment in the biological samples. In this study, we explored two-photon optical tomography of human skin specimens using two-photon excited autofluorescence imaging combined with FLIM by using a femtosecond laser (Coherent Mira900F), a laser scanning confocal microscope (Leica TCS SP2) and a time-correlated single-photon counting module (Becker & Hickl, SPC 150) which is coupled to the optional port of the confocal system. In stained human skin specimens, the nonlinear induced autofluorescence is generated from dye fluorophores. In unstained samples, there are a number of naturally endogenous fluorophores in skin sample, such as keratin, melanin, collagen, elastin, flavin and porphyrin. Confocal microscopy was used to obtain structures of the sample. Properties of epidermic and cancer cells were characterized by fluorescence emission spectra, as well as fluorescence lifetime imaging. In conclusion, the noninvasive two-photon autofluorescence lifetime imaging method has provided accurate optical biopsies with subcellular resolution, suggesting a better quantitative optical diagnostic method in skin cancer diagnosis.

8948-87, Session PSun

Simultaneous selective two-photon microscopy using MHz rate pulse shaping and quadrature detection of the time-multiplexed signal

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The near octave-spanning oscillator and pulse shaper (femtoAdaptiv, BioPhotonic Solutions Inc.) with compensation phase mask applied produces sub-10 fs laser pulses with central wavelength at 812 nm at 81 MHz repetition rate, making it capable of inducing two-photon excitation in the 380 to 500 nm range. The output is split into two arms with different second order dispersion (SOD). The recombined beams create a train of pulses with phase-shape switching at 162 MHz rate. Each pulse induces selective TPEF on the sample at wavelengths determined by the amount of SOD in the beam, which tunes the selective TPEF wavelength. Fluorescence is detected by single fast photomultiplier tube (PMT) detector; therefore, signal from the PMT detector contains fluorescence signals from two different selectively-excited fluorophores. The two separate signals are isolated by quadrature detection using a lock-in amplifier. Images are obtained from the two different fluorophores simultaneously at 81MHz. The wide tunability of the two-photon excitation wavelength, fast switching rate between the selective excitation and low photodamage (due to low power of laser beam) enables potential application of this method for in vivo dynamic imaging in biological samples.

8948-88, Session PSun

Intravital imaging of kidney pathology using two-photon microscopy and optical coherence tomography

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Adriamycin (doxorubicin), a common cancer chemotherapeutic drug, is used to induce a model of chronic progressive glomerular disease in rodents. In our studies, we evaluated renal function changes in a rat model after Adriamycin injection using non-invasive multimodal intravital imaging techniques including two-photon microscopy (TPM), optical coherence tomography (OCT) and Doppler OCT (DOCT).

We injected Adriamycin (1.5mg/kg) into adult male Munich-Wistar rats to induce changes that simulate glomerular sclerosis in humans. With the advantages of deep penetration and fast frame scanning speed for three-dimension (3D) label free imaging, OCT/DOCT system was able to quantify glomerular volume and reflectivity, tissue texture, and renal hemodynamics in the glomeruli. With the advantages of high resolution and deep penetration imaging, we developed an intravital TPM system to quantify the tubular volume and diameter. Glomerular permeability and filtration, proximal and distal tubules, and capillary hemodynamics were evaluated by intravenous infusion of fluorophore-labeled dextrans. Immediately following TPM, these kidneys were preserved by perfusion-fixation, sectioned and stained with hematoxylin and eosin (H&E) in order to compare our observations.

The procedures of combined TPM and OCT/DOCT imaging revealed tubular atrophy, tubular distention, glomerular sclerosis and hyaline casts in the distal nephron as early as 1-2 weeks after injection. These changes become progressively more severe over the next 4-8 weeks. Our results demonstrate the strong potential and promise for the application of combined TMP, OCT and Doppler OCT in kidney pathology, as it provides a comprehensive examination of the kidney function, in real time, both visually and quantitatively.

8948-89, Session PSun

Trends in cytosolic lipid content for mammalian oocytes investigated by coherent anti-Stokes Raman scattering (CARS) microscopy

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Lipid biology is a fast growing field with its research exploring such topics including its roles in cellular homeostasis, metabolism, and membrane regulation. Lipids have a heavy influence on mammalian oocytes and subsequent preimplantation embryos where they have been found to have both beneficial roles in their use as an endogenous energy source, as well as detrimental effects in cryopreservation efficiency. To this end, we have implemented hyperspectral imaging using broadband coherent anti-Stokes Raman scattering (CARS) microscopy to investigate correlations between cytosolic lipid content (CLC) and oocyte growth, development, and vitrification. Image analysis was performed using singular value decomposition (SVD) on CCD-based hyperspectral images, and intensity thresholding on PMT-based images. Oocytes were used with IRB or UCUCA approval, all statistics were performed using Student's t-test, and all experiments were supplemented with

fluorescence microscopy on fixed/stained oocytes. Proof-of-concept studies with oocytes from species with known differences in CLC demonstrated that CLC was highest in porcine, moderate in bovine, and lowest in murine and human. In murine oocytes, imaging two developmentally distinct states and two meiotic maturation stages showed an increase in CLC during cell growth, followed by a decrease as oocytes entered meiosis. A decrease in CLC was observed in cryopreserved bovine oocytes, and CLC was partially retained using a novel microfluidic vitrification device. In conclusion, broadband CARS represents a non-invasive, live-cell, real-time method of lipid analysis in mammalian oocytes. These results suggest an importance of lipids in oocyte biology, and further facilitate CARS as a noninvasive clinical tool.

8948-90, Session PSun

Fast multiplexed time-resolved fluorescence microscopy for quantitative time-lapse FRET imaging in cells and deep tissue

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Fluorescence lifetime imaging microscopy (FLIM) allows quantitative FRET analysis that detects molecular interactions in live biological specimen. We report a novel FLIM-FRET method, named Fourier lifetime excitation-emission matrix (FLEEM) confocal microscopy, which performs high speed 3D imaging at multiple excitation-emission channels in parallel. Operating under the principle of Fourier multiplexed lifetime spectroscopy [1], FLEEM simultaneously acquires multiplexed confocal lifetime images at multiple excitation-emission wavelength combinations at 44,000 pixels/sec. It produces time-resolved images in the form of excitation-emission matrix (EEM), which enables robust characterization of FRET through the complete analysis of all possible photon-pathways in the FRET phenomenon. The time-resolved EEM analysis extracts information from additional photon-pathways not accessible with conventional FLIM-FRET methods that only measures fluorescence decay of donor, and allows simple and robust quantification of FRET without extensive computing with global analysis. The method was first demonstrated with time-lapse confocal imaging of live HeLa cells expressing TagBFP-D2-GFP FRET calcium sensor. FRET efficiency of the bound and unbound states of the sensor was extracted by the time-resolved EEM analysis, which enables the conversion of donor fluorescence lifetime images to calcium concentration level images. The method was then applied to deep-tissue tomography imaging of live zebrafish embryos, in which calcium and cAMP levels are simultaneously observed through multiplexed FLIM-FRET. Our work opens the door to multiplexed FLIM-FRET and quantitative investigation of multiple biochemical interactions in live cells and tissues.

[1] M. Zhao and L. Peng, Optics Letters. 35,2910. (2010)

8948-91, Session PSun

In situ dissolution analysis of pharmaceutical dosage forms using coherent anti-Stokes Raman scattering (CARS) microscopy

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A custom-built intrinsic flow-through dissolution setup was developed and incorporated into a home-built CARS microscope consisting of a synchronously pumped optical parametric oscillator (OPO) and an inverted microscope with a 20X/0.5NA objective. CARS dissolution images (512x512 pixels) were collected every 1.12s for the duration of the dissolution experiment. Hyperspectral CARS images were obtained pre- and post-dissolution by rapidly imaging while sweeping the wavelength of the OPO in discrete steps so that each frame in the data stack corresponds to a vibrational frequency. An image-processing routine projects this hyperspectral data into a single image wherein each compound appears with a unique color.

Dissolution was conducted using theophylline and cimetidine-naproxen co-amorphous mixture. After 15 minutes of theophylline dissolution, hyperspectral imaging showed a conversion of theophylline anhydrate to monohydrate, confirmed by a peak shift in the CARS spectra. CARS dissolution images showed that monohydrate crystal growth began immediately and reached a maximum with complete surface coverage at about 300s. This result correlated with the UV dissolution data where surface crystal growth on theophylline tablets resulted in a rapidly reducing dissolution rate during the first 300s. Co-amorphous cimetidine-naproxen cracked during dissolution at pH 7 while this was not observed at lower pH and neither for the individual crystalline components.

We observed solid-state conversions on the tablet's surface in situ during dissolution. Hyperspectral CARS imaging allowed visual discrimination between the solid-state forms on the tablet's surface. In the case of theophylline we were able to correlate the solid-state change with a change in dissolution rate.

8948-92, Session PSun

Annular beam-shaping for two-photon fluorescence microscopy: an investigation of the effect of annular beam-shaping on the point-spread-function in two-photon fluorescence microscopy

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Two-photon fluorescence microscopy (TPM) is a well-established method for high resolution, non-invasive investigations of biological tissue. The low probability of the two-photon excitation process ensures that the detected signal is only generated within the focal volume where the photon flux is high; however, out-of-focus fluorescence has been demonstrated to be a limiting factor. We here explore annular beam shaping for TPM. Annular beams are expected to reduce background fluorescence, preferential for deep tissue imaging. Annular beams will create Bessel beams which will carry special characteristics, e.g. elongated focal volume. In order to correctly implement annular beam shaping in TPM it is necessary to have full control on the shape and size of the focal volume, i.e. the point spread function (PSF).

In this study we investigate how the outer/inner diameter ratio of the beam affects the laser distribution. Simulations are performed by implementing the Fresnel–Kirchhoff diffraction integral in MATLAB. The simulations demonstrate that the focal area resembles that of an ordinary Gaussian beam for an inner radius of up to 40-50% of the full radius, when using an objective lens in the NA-range of 0.8 to 1, thus preserving axial resolution while lowering the out-of-focus signal. When the inner radius of the beam exceeds this value, the focal volume becomes axially elongated. Measurements are presently undertaken on a custom-built TPM set-up to confirm the data experimentally. The study implies that annular beam-shaping using a medium inner radius can be applied for conventional TPM, particularly for deep tissue imaging.

8948-93, Session PSun

Fluorescence lifetime imaging microscopy using a streak camera

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Fluorescence microscopy has become an important tool in biomedicine. Like fluorescence spectrum, fluorescence lifetime is a characteristic of a given fluorescent molecule and can be used for distinguishing different molecular species in a sample and contrasting different fluorophores. Moreover, fluorescence lifetime is very sensitive to the microenvironment of the fluorophores, i.e. a change in the fluorescence lifetime of a fluorophore reflects a change in its local environment. Thus a lot of physiological parameters including pH, Ca2+, Na+ and pO2 can be quantified from fluorescence lifetime measurement. Fluorescence lifetime imaging microscopy (FLIM) combines time-resolved fluorescence spectroscopy to imaging microscopy and aims to analyze quantitative parameters of fluorescence at a cell or tissue level. Generally, there are three main implementation methods for FLIM, including time-gated image intensifier, time-correlated single-photon counting (TCSPC) and streak camera. In this paper, we present the development of fluorescence lifetime imaging microscopy system based on a streak camera (streak-FLIM), which couples ultrafast infrared laser for multiphoton excitation and a streak camera for lifetime measurements. The streak-FLIM system was calibrated with an F-P etalon and several standard fluorescent dyes. Preliminary experimental results on fluorescence lifetimes of plant leaves are obtained. The streak-FLIM system may have potential applications in the diagnosis of cancer tissue and fluorescence resonance energy transfer (FRET) imaging of living cells.

8948-94, Session PSun

TCSPC-based phosphorescence lifetime spectroscopy and imaging with metal complexes

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The characterization of phosphorescent compounds has been for many years of great importance in the field of materials science namely chemical sensing, and has renewed its interest over the past decade with the booming development of organic light emitting diode (OLED) technology.

For nanosecond excited state lifetimes time-correlated single photon counting (TCSPC) with picosecond timing has become already a key method over the past years in biophysics and the life sciences. Based on a newly developed TCSPC platform [1] we are now able to cover lifetimes from picoseconds up to milliseconds, combined with a dramatically reduced dead time to allow for efficient multistop photon detection after pulsed excitation. The all-in-one device philosophy allows also for simultaneous fluorescence and phosphorescence detection and can in a straightforward way be combined with rasterscanning to enable phosphorescence lifetime imaging. This paves the way to phosphorescence spectroscopy and imaging with common TCSPC equipment.

Many phosphorescent compounds are based on metal complexes with organic ligands. Such ligands, when unbound to the metal, display quick fluorescence. We will show that combined fluorescence and phosphorescence, even in the same decay measurement, have a great potential for accurate and quick characterization of these metal complexes and their usage. Further we started to evaluate TCSPC based phosphorescence imaging to measure oxygen consumption in living cells monitored by transition metal complexes.

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8948-95, Session PSun

Integrated coherent Raman scattering and multiphoton microscopy for label-free imaging of the tooth

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We report the implementation of a unique multimodal nonlinear optical microscopy (i.e., stimulated Raman scattering (SRS), coherent anti-Stokes Raman scattering (CARS), second-harmonic generation (SHG), third-harmonic generation (THG) and two-photon excitation fluorescence (TPEF)) platform developed for label-free imaging of the tooth. A picosecond tunable laser together with an OPO system is used as an excitation source for simultaneously multimodal imaging. The difference in collagen contents in different regions of the dentin was demonstrated by polarized SHG. CARS image is used to selectively excite the collagen in dentine and organic matrix in enamel. CARS imaging gives clear biochemical distributions across the tooth with a better optical sectioning ability compared to TPEF imaging. SRS can be set to either image the enamel or the organic matrix in enamel and collagen. Compared with CARS, a higher chemical specificity was achieved using SRS because of free of non-resonant background interference. The detailed structure of enamel was more clearly visualized by the combination of THG and SRS imaging. This work demonstrates that combining different nonlinear optical imaging modalities provides new insights into the understanding of morphological structures and biochemical/biomolecular distributions in the dentine and enamel of the tooth without the need of labeling, paving the way for early diagnosis and characterization of tooth disease in dentistry at the submicron level.

8948-96, Session PSun

Integrated optical coherence and multiphoton microscopy for in vivo assessment of engineered skin substitutes

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As the body's first line of defense against the external environment, skin plays a critical role in human health. Loss or damage of a major portion of skin can be disabling and potentially life threatening. There is a high demand for engineered skin substitutes due to the large number of cases in which autologous skin grafts cannot be performed due to wound size or location. In this study, we demonstrate the application

of an integrated intravital optical coherence (OCM) and multiphoton (MPM) microscope capable of acquiring time-lapse co-registered images to study engineered skin-assisted wound healing in mice with green fluorescent protein- (GFP) encoded bone-marrow (BM) derived cells. Through a combination of modalities including OCM, two-photon excitation fluorescence (TPEF) microscopy, second harmonic generation (SHG) microscopy, and phase variance optical coherence tomography (OCT), we are able to noninvasively track many of the critical parameters of wound healing including structural remodeling, BM derived stem and immune cell dynamics, collagen synthesis, and angiogenesis. Using this in vivo integrated imaging approach, we investigate the effectiveness of skin substitutes comprised of porous alginate hydrogels that are used to deliver allogeneic dermal fibroblasts to wounds in GFP BM mice. Through the use of this integrated imaging technique, we are able to more fully characterize the wound healing process as well as the efficacy of engineered skin substitutes. This work demonstrates the significance of an integrated approach, combining structural and functional optical imaging, for extracting clinically relevant data that is important for assessing engineered skin substitutes in vivo.

8948-97, Session PSun

Characterizing heterogeneity in single adipocytes using stimulated Raman scattering microscopy and transcriptome analysis

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During adipogenesis, cells accumulate fatty acids in intracellular lipid droplets as they differentiate into fat cells. Adipocyte differentiation is a fundamental process in obesity and related pathologies such as diabetes and heart disease. Lipid droplet formation during adipogenesis is regulated by the expression of adipogenic genes yet mature adipocytes show significant heterogeneity in lipid droplet distribution. Correlation between lipid droplet morphology and gene expression in differentiating adipocytes can reveal important and complex gene regulatory networks that control adipogenesis and lipid metabolism, however population measurements can often obscure these relationships because of the large cell to cell variation in gene expression and lipid droplet morphology. We combine, single cell transcriptome analysis, stimulated Raman scattering microscopy, and microfluidics to characterize phenotypic heterogeneity in single cells during adipogenesis. The integration of microfluidic control allows us to sort single cells and measure the correlation between lipid droplet distribution and gene expression within the same single cell.

8948-98, Session PSun

Rapid acquisition of lipid distribution in C. elegans by stimulated Raman scattering (SRS) microscopy

Tao Chen, Ang Li, Aaron M. Streets, Yanyi Huang, Peking Univ. (China)

As a basic construction component and the functional molecules in life, lipids are involved in many biological activities. Lipid research can help to reveal mechanisms in metabolism and functionality in different levels from cell to individual. Caenorhabditis elegans is the most widely used animal model in lipid research. We developed a high-throughput method to rapidly scan C. elegans to identify lipid distribution in live animals. We used a microfluid-based device to manipulate worms and imaged them with simulated Raman scattering (SRS) microscopy. With this method, one could handle large quantities of worms within a short time compared with conventional techniques. Lipid-related gene screening can also be achieved with this method.

8948-99, Session PSun

Polarization-maintaining dichroic module for excitation polarization-dependent multiphoton microscopy

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In excitation polarization-dependent (EPD) multiphoton microscopy, the polarization state of the excitation light beam(s) needs to be conserved in a microscopy system in order to assess the molecular structure of biological samples in a meaningful way. Most difficulties in achieving an unaltered polarization state at the sample arise because of both dichroism and ellipticity introduced by a primary dichroic (PD) filter located upstream from the microscope objective. The presence of this dichroic in epi-detection microscopy systems is necessary, and is used to separate excitation light from signal originating from the sample. In this work, we propose a novel polarization-maintaining dichroic module (PMDM) that can be used to compensate for any alterations to the polarization state of light introduced by the PD. The compensating effect of the proposed module works simultaneously and equally well for light beams of different wavelengths, and is independent of the initial polarization state of the uncompensated light beams. In order to fully demonstrate the utility of the PMDM for polarization compensation, we perform EPD second-harmonic generation microscopy of fibrils of type I collagen from rat tail tendons, EPD two-photon excited fluorescence imaging of myelin sheaths stained with nile red and EPD coherent anti-Stokes Raman scattering microscopy of unstained myelin sheaths. Given that the molecular structure of biological samples might prove to be an early biomarker for disease pathologies, it is of great interest to better constrain the polarization state of light used to probe a sample.

8948-100, Session PSun

3D and time-lapse FLIM images of a Parhyale hawaiensis embryo development from one cell stage

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The understanding of stem cell differentiation could mean total control over diseases and creation of artificial organs or lifeforms for several purposes. The best samples to study stem cells are embryos. Moreover, the great similitude among different species, means that one could use almost any embryo as a model to understand cell differentiation. We discovered an almost ideal embryo, Parhyale hawaiensis (P.h.), to be observed under Non Linear Optics (NLO) confocal microscopy because it is small, around 200 microns, which fits in the field of view, well protected against bacteria inside the egg shell, short development time, transparent and the fertilized eggs can be removed from females with a needle prior to their first cleavage, in the single cell stage. P.h. is an amphipod crustacean that has been widely used as an important model in evolutionary, developmental and genetic analysis. Its embryonic process was completely described and its genome studied. In this

paper, we observed a Parhyale embryo developing with different NLO techniques in 3D and in a time lapse mode. To do that we have used an integrated multimodal Non-Linear Optics platform, and acquired images of two-photon excited fluorescence, THG and FLIM techniques. For that we faced some challenges on the acquisition of sets of z-stack FLIM images in a time-lapse mode to reconstruct a 3D FLIM movies of the embryo developing. Our results confirm it can stands for prolonged periods exposed to fs laser beams and that THG and 3D time lapse FLIM images sequences can be obtained.

8948-101, Session PSun

Hyperspectral imaging via spectral interferometric polarised coherent anti-Stokes Raman scattering

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Quantitative broadband coherent Raman imaging of biological samples, analogous to spontaneous Raman microspectroscopy, requires the removal of the non-resonant background (NRB) for interpretation of vibrational energies and the detection of weak resonances. Previous experimental approaches for removing the NRB tend to be instrumentally difficult, have stringent requirements on laser properties, or require assumptions about the NRB. Computational approaches produce good approximations to spontaneous Raman spectra if the spectra are of sufficient width, but also intrinsically produce a spectrally varying error signal, and require estimation of the spectral variation of the Stokes field by measuring the NRB in a reference material. This is itself often compromised by a residual resonant response, and changes of the Stokes spectrum during an acquisition leads to errors that can mask the weaker resonances in the fingerprint region of biological samples.

We have developed a new method for quantitative broadband CARS spectral imaging, which uses passive polarisation optics combined with spectrally-resolved balanced homodyne detection. The NRB is removed in a single exposure, without requiring an independent measurement of the Stokes spectrum. The resulting spectra are amplified by the non-resonant response, vary linearly with concentration, and can be directly related to polarised spontaneous Raman spectra. The technique has relaxed requirements on spectral phase and instrument stability, and is suitable for any laser system capable of generating CARS. We have acquired spectra, with high signal-to-noise ratio, down to exposures of 10 ms, and have successfully applied the method to rapid hyperspectral Raman imaging.

8948-102, Session PSun

Combined second-harmonic generation and sum-frequency generation microscopy reveals the chemical origin of the second-order optical response of collagen

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Sum frequency generation (SFG) and second harmonic generation (SHG) are nonlinear optical techniques that probe the second-order nonlinear susceptibility of materials. Although SHG has been widely used in microscopy, the SHG signal is nonresonant, which obscures a direct link between the optical signal and the chemical moiety responsible for the second-order molecular response. In comparison, vibrationally sensitive SFG can be tuned into direct resonance with selected vibrational modes that underlie the optical nonlinearity. SFG shares with SHG microscopy the advantages of three-dimensional focusing, fast imaging, high spatial resolution, and selectivity to noncentrosymmetric molecular ordering. In an effort to clarify the chemical origins of the contrast seen in SHG imaging of collagen, we have built a microscope for the simultaneous acquisition of nonresonant SHG and vibrationally resonant SFG signals. Relative to bulk spectroscopy measurements, the advantage of performing combined SHG and SFG in a microscope is that the imaging mode provides unambiguous information about molecular alignment on a sub-micrometer scale. We utilized this new nonlinear microscope to study collagen I in rat tail tendon and hawk cornea. By using polarization sensitive measurements as a function of vibrational energy, we have found that selected methylene modes of collagen constitute a major contribution to the SHG signal. A detailed analysis of our imaging results reveals an enhanced picture of the various off-resonant contributions to the SHG signal, which adds chemical relevance to SHG microscopy.

8948-103, Session PSun

Investigating backward scattered second harmonic generation from various mouse collagen tissues

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The backward scattering of forward Second Harmonic Generation (BS-SHG) is an important source of backward collected SHG. Quantification of BS-SHG can lead to better understanding of the mechanism of backward SHG and improvement on the signal collection in SHG imaging and spectroscopy. In this paper, we use a confocal multiphoton system with various detection pinholes to differentiate BS-SHG from backward generated SHG (BG-SHG) based on the fact that BS-SHG is more scattered and therefore has a much bigger spot size than BG-SHG. BS-SHG is quantified from various types of mouse tissues, such as Achilles tendon, tail tendon and skin, and at various focal depths. It is found that the proportion of the BS-SHG to the total backward collected SHG varies from 13.8% to 62.9%, and the proportion of the BS-SHG to the forward generated SHG varies from 2.0% to 7.38%, in the mouse tail tendon, back skin, and Achilles tendon, respectively. When the focal point is moved deeper into tissue, the contribution of BS-SHG is found to decrease due to a reduced pass length of the forward photons. A Monte Carlo simulation is conducted which quantitatively verifies the experimental results. In addition, other SHG characteristics such as the Forward/Backward (F/B) intensity ratio and the excitation wavelength dependence are also investigated. It is observed that Achilles tendon has a higher F/B ratio as well as a higher BS-SHG contribution than the skin and tail tendon. This correlation indicates that the SHG scattering and generation are likely affected by the similar microstructures of collagen fibrils inside and outside of the focal volume. By investigating the BS-SHG, F/B ratio and SHG excitation spectrum, a more comprehensive understanding about SHG generation and scattering in tissue can be obtained.

8948-104, Session PSun

Signal-to-noise ratio improved in resonant scanning system by adding pulse splitter

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Abstract: Two-photon fluorescence excitation (TPE) microscopy has been an important image tool for In Vivo study. To capture fast event, video rate imaging techniques such as resonant scanner has been developed. However, at video rate imaging (30 frames per second) the pixel dwell time is reduced to about 100ns. As the result, the two photon excitation per pixel is very low therefore signal per pixel is very poor. To get better

image, there are several methods have been used such as increasing laser power, averaging over several frames. However, increasing laser power could induce photo-damage and averaging over frames actually decreases the imaging speed. Here, we combined a passive pulse splitter to improve the signal without scarify the imaging speed by increasing excitation event. Our results showed that the signal is significantly improved for video rate imaging with 8 pulses splitter. In addition, the Signal to Noise ratio is also increased so that we can achieve quality image without losing speed.

8948-105, Session PSun

Pulse splitter-based nonlinear microscopy for live-cardiomyocyte imaging

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Cardiac hypertrophy, accompanied with sarcomeric addition, is an adaptive response to hemodynamic overloads. Understanding the detailed process and the location of the sarcomeres that are added to the existing ones is important to the study of the early development of pathological cardiac hypertrophy. Neonatal rat cardiomyocyte culture is a very useful model for the study of sarcomeric addition because during the cell spread, new sarcomeric additions are visualizable through second harmonic generation (SHG) microscopy. However, during the early stage of cell culture in which sarcomeric additions occur, the neonatal cardiomyocytes are very sensitive to photo-damage. Due to high rate of cell death, the sarcomeric addition cannot be systematically studied using conventional SHG system. To address this challenge, we have successfully introduced the pulse-splitter system developed by Ji's research group into our two photon excitation fluorescence (TPEF) and SHG microscope, which has been shown to be an idea system to observe sarcomeric additions in developed neonatal cardiomyocyte cultures. Our TPEF-SHG microscope is a lab-built system with a 60X water immersion objective lens (NA = 1.0) that enabled clear visualization of 3D structure of sarcomeres in vitro. The pulse splitter is established by splitting one pulse with partially reflective interface multi-times and then recombining all differentially delayed sub-pulses into one set of pulse trains. By doing this, the pulse repetition rate of the Ti-sapphire femtosecond laser can be increased up to 128 times of its original value. Dramatic improvement of cell viability was achieved by using the pulse-splitting technique. Consequently, morphology changes of the myofibrils at various culturing stages were recorded while the freshly cultured neonatal cardiomyocytes were spreading on the culturing substrate.

8948-106, Session PSun

Investigations on the biological samples by using different techniques in apertureless near-field optical microscopy

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Scanning near-field optical microscopy (SNOM) is one technique that offers the possibility to investigate optical properties with sub-wavelength resolution. In near-field optical techniques, the basic idea is to access the evanescent fields of a structure or to couple evanescent fields from a light source to a structure under investigation. Aperturelss SNOM can be used to investigate the near field created at the sample surface (scattering- SNOM) or to image the optical second harmonic generated in SNOM. Our work was focused on the investigations of the biological samples by using the both of the techniques. We used an apertureless SMOM which upgraded an atomic force microscope. The details regarding the surface topography were also obtained. In the frame of all techniques the resolution connected with the tip size was less than 10 nm.

8948-107, Session PSun

Determining the diffusion coefficient of fluorescent beads through phasor-FLIM

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FLIM or fluorescence lifetime imaging microscopy [1] traditionally provides a versatile tool for spatially-mapping fluorescence lifetimes and macromolecular interactions [2] through pixel-by-pixel resolution of the excited-state lifetime.

It is possible to show that FLIM can also measure lateral motions. In conventional frequency-domain FLIM the phase and modulation of the detected fluorescence are determined by the photophysics of the fluorophore only. However, translational motion on the timescale of FLIM acquisition can significantly perturb apparent phase and modulation values owing to intensity fluctuations. This perturbation can be visualized most conveniently in phasor plot [3, 4].

In FLIM experiment, we focus on motions that cause large intensity fluctuations on the timescale of image acquisition. A simple analytic theory, numerical simulations and measurements on fluorescent beads by means of the phasor plot is defined. Fluctuations due to particle motions increase the number of data points on phasor plot and their area of aggregation, an effect we refer to as phasor broadening. We relate the phasor broadening to diffusion coefficient of beads. The results exhibit a new application of FLIM for detecting and determining translational motions.

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8948-22, Session 4

Vibrational imaging of newly synthesized proteins in live cells, tissues, and animals by stimulated Raman scattering microscopy *(Invited Paper)*

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De novo protein synthesis plays an indispensable role in biological systems by regulating cell responses to various environmental stimuli in both physiological and pathological conditions. However, visualization of newly synthesized proteome in live systems with subcellular resolution has been proven to be highly challenging despite the extensive efforts along the lines of fluorescence staining, autoradiography, and mass spectrometry. Herein, we report a novel live-cell imaging technique to visualize nascent proteins by harnessing the emerging stimulated Raman scattering (SRS) microscopy coupled with metabolic incorporation of deuterium-labeled amino acids. SRS microscopy, a nonlinear vibrational imaging technique, offers distinctive advantages including Raman

amplification, bond-selectivity, background-free and biocompatibility, whereas the introduction of deuterium as an exogenous label is minimally perturbative to proteins. Thus SRS imaging of carbon–deuterium bonds (C–D) in the cell-silent Raman region is highly sensitive, specific, and compatible with living systems. We demonstrate our technique by imaging newly synthesized proteins in live cancer cell lines, primary neurons, brain tissue slices and animals under a fast image acquisition speed. Subcellular compartments with fast protein turnover are clearly identified in the resulting spatial maps of the quantitative ratio between new and total proteomes. Thus, nonlinear vibrational imaging of stable isotope incorporation will be a valuable tool for studying the complex spatial and temporal dynamics of newly synthesized proteome in vivo.

Wei L, Yu Y, Shen Y, Wang MC and Min W. Proc. Natl. Acad. Sci. USA 110(28), 11226 (2013).

8948-23, Session 4

Spectroscopic imaging unveils the essential role of cholesterol accumulation in cancer proliferation *(Invited Paper)*

Ji-Xin Cheng, Shuhua Yue, Junjie Li, Seung-Young Lee, Purdue Univ. (United States); Liang Cheng, Indiana Univ. (United States); Timothy Ratliff, Xiaoqi Liu, Purdue Univ. (United States)

No Abstract Available

8948-24, Session 4

Stimulated Raman scattering microscopy of human brain tumor specimens

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The central goal of brain tumor surgery is to remove as much tumor as possible without disrupting normal adjacent brain. Unfortunately, detecting tumor infiltration during surgery is a challenge and leads to inconsistent outcomes. Here we report the use of stimulated Raman scattering (SRS) microscopy as a label-free means of differentiating healthy brain from tumor-infiltrated brain in human surgical specimens. In thinly-sectioned, flash-frozen human specimens, SRS demonstrates the key features of gray matter, white matter, and tumor infiltrated brain that are commonly imaged with hematoxylin and eosin staining. SRS also delineates normal- from tumor-infiltrated brain in fresh, unprocessed surgical specimens. SRS accurately depicts the diverse histologic differences between intra- and extra-axial brain tumors. These results support the translation of SRS as an imaging modality that could be used to guide surgery based on the microscopic differences between healthy and tumor-infiltrated brain.

8948-25, Session 4

Broadband hyperspectral coherent anti-Stokes Raman scattering microscopy for stain-free histological imaging with principal component analysis

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Routine procedures in standard histopathology involve laborious steps of tissue processing and staining for final examination. New techniques which can bypass these procedures and thus minimize the tissue handling error would be of great clinical value. Coherent anti-Stokes Raman scattering (CARS) microscopy is an attractive tool for label-free biochemical-specific characterization of biological specimen. However, a vast majority of prior works on CARS (or stimulated Raman scattering (SRS)) bioimaging restricted analyses on a narrowband or well-distinctive Raman spectral signatures. Although hyperspectral SRS/CARS imaging has recently emerged as a better solution to access wider-band spectral information in the image, studies mostly focused on a limited spectral range, e.g. CH-stretching vibration of lipids, or non-biological samples. Hyperspectral image information in the congested fingerprint spectrum generally remains untapped for biological samples.

In this regard, we further explore ultrabroadband hyperspectral multiplex (HM-CARS) to perform chemoselective histological imaging with the goal of exploring its utility in stain-free clinical histopathology. Using the supercontinuum Stokes, our system can access the CARS spectral window as wide as >2000cm-1. In order to unravel the congested CARS spectra particularly in the fingerprint region, we first employ a spectral phase-retrieval algorithm based on Kramers–Kronig (KK) transform to minimize the non-resonant background in the CARS spectrum. We then apply principal component analysis (PCA) to identify and map the spatial distribution of different biochemical components in the tissues. We demonstrate chemoselective HM-CARS imaging of a colon tissue section which displays the key cellular structures that correspond well with standard stained-tissue observation.

8948-26, Session 4

High-speed, broadband coherent Raman imaging (CRI) of glioblastomas using broadband coherent anti-Stokes Raman scattering microspectroscopy

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Glioblastoma multiforme is an aggressive primary brain tumor with a median survival rate of less than 2 years. Typical optical analysis of tissue specimens uses time-consuming dyes and stains that provide limited molecular information. To address the need for high-speed, label-free chemical imaging , we have developed a broadband coherent Raman imaging microscope capable of acquiring Raman spectra over 600 – 3600 cm-1 with high signal-to-noise. Using this system we are able to analyze a variety of biological tissues, including de novo and xenograft brain tumors, with dwell times of 3.5 ms without the need for frame averaging.

The microscope uses broadband coherent anti-Stokes Raman scattering to probe the intrinsic Raman vibrational energy levels within the sample. This microscope uses two co-seeded fiber lasers: a 3.4 ps probe to provide high spectral resolution and a 16 fs supercontinuum (SC) that spans over 400 nm. At higher Raman energies, vibrations are stimulated with a degenerate pump-probe ("2-color" excitation) that also generates a modest nonresonant background (NRB) (methanol sensitivity: < 8.0 mM). At lower Raman energies, the SC provides the pump and Stokes photons ("3-color" excitation) and generates a tremendous NRB that acts as a heterodyne amplifier for the weak fingerprint peaks. For example, from within the nucleus of an endothelial cell (single 660 x 660 nm pixel), we can recover 5 peaks emanating from DNA just between 668-832 cm-1. Using this technology, we have analyzed several tissues from healthy

mice as well as xenograft and de novo glioblastoma murine model systems.

8948-27, Session 5

Combining fluorescence and CARS microscopy to uncover cellular biomarkers of multiple sclerosis *(Invited Paper)*

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By combining multiple optical modalities such as fluorescence and Raman scattering into an in vivo microscope, we can image the many players involved in the early development of multiple sclerosis-like lesions in mice. By looking at dynamic blood vessel permeability, cellular infiltration of myeloid cells, and myelin damage in the spinal cord, we can identify start to obtain a better picture of the early stages of this disease. We identify that vessel permeability to different types of molecules follows different dynamics and may enable cellular entry in the central nervous system, leading to myelin damage.

8948-28, Session 5

CARS microscopy of Alzheimer's diseased brain tissue *(Invited Paper)*

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder currently without cure, characterized by the presence of extracellular plaques surrounded by dystrophic neurites1. In an effort to understand the underlying mechanisms, biochemical analysis (protein immunoblot) of plaque extracts reveals that they consist of amyloid-beta (A?) peptides assembled as oligomers, protofibrils and aggregates1. Their spatial distribution has been confirmed by Thioflavin-S or immuno-staining with fluorescence microscopy. However, it is increasingly understood that the protein aggregation is only one of several mechanism that causes neuronal dysfunction and death. This raises the need for a more complete biochemical analysis. In this study, we have complemented 2-photon fluorescence microscopy of Thioflavin-S and A? immuno-stained human AD plaques with CARS microscopy and preliminary Raman microspectroscopy. We show that the chemical build-up of AD plaques is more complex and that A? staining does not provide the complete picture of the spatial distribution or the molecular composition of AD plaques. CARS images provide important complementary information to that obtained by fluorescence microscopy, motivating a broader introduction of CARS microscopy in the AD research field.

8948-29, Session 5

Simultaneous stimulated Raman scattering and higher harmonic generation imaging for liver disease diagnosis without labeling

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Nonlinear optical microscopy (e.g., higher harmonic (second-/third-harmonic) generation (HHG), simulated Raman scattering (SRS)) has high diagnostic sensitivity and chemical specificity, making it a promising tool for label-free tissue and cell imaging. In this work, we report a development of a simultaneous SRS and HHG imaging technique for characterization of liver disease in a bile-duct-ligation rat-modal. HHG visualizes collagens formation and reveals the cell morphologic changes associated with liver fibrosis; whereas SRS identifies the distributions of hepatic fat cells formed in steatosis liver tissue. This work shows that the co-registration of SRS and HHG images can be an effective means for label-free diagnosis and characterization of liver steatosis/fibrosis at the cellular and molecular levels.

8948-30, Session 5

Imaging protein misfolding in Alzheimer's disease with SRS microscopy

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One of the major pathological hallmarks of Alzheimer's disease (AD) is the aggregation of misfolded amyloid beta peptide into senile plaques. The fibrous plaques have distinct conformational state, very rich in beta sheet. We discovered that stimulated Raman scattering (SRS) imaging of tissue in the Amide I region allows us to detect amyloid plaques from normal tissues. Amide I vibration is highly sensitive to protein conformations, its Raman spectra differs between normal protein and beta-sheet rich amyloid plaques. Such spectral differences have enabled multi-color SRS microscopy to image amyloid plaques in a label-free manner. SRS microscopy provides a new method to study AD, which complements the traditional staining methods.

8948-31, Session 5

CARS microscopy of cancer cells in vitro and tumors in vivo

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Identification of cancer cells in tumors is still a challenge in the biomedical field. Coherent Anti-Stokes Raman Scattering (CARS) microscopy might be useful for fast retrieval of 3D imaging as it is a label-free imaging technique that is capable of real-time, non-pertubative examination of living cells and organisms based on molecular vibrational spectroscopy with a sub-micron spatial resolution.

In this effort, we have taken CARS and Second Harmonic Generation (SHG) images of epithelial and cancer cells in cultures and in tumor tissues using an optical setup developed in collaboration with Genia Photonics. Observations were performed with a pump bean at 817 nm and a stokes beam at 1065nm for resonance and 1080nm for off resonance.

Cells were embedded in collagen gel and cultures were observed directly on a microscopic platform. 3D morphology of individual cells was seen as well as cell assemblies in 3D imaging. 3D video reconstructions of resonance images showed areas of gel biodegradation around cancer cells. Cell morphology was close to that seen by phase contrast microscopy. Lipids were seen all over the cytoplasmic membrane and in lipid vesicles and they were highly present at delimiting cell borders by contrast with the non-resonance CARS imaging.

Tumor xenografts lymphoid tissue-grafted tumors were induced after

injection of cancer cells in mouse lymphoid tissue and thick cryosections of label-free tissues were observed in CARS and SHG microscopy. CARS imaging of the tumors distinguished clearly cancer cells from the inflammatory and fibrotic reaction (as observed with SHG) as well as from the normal lymphoid tissue.

8948-32, Session 5

Investigating cold-induced injury of sebaceous glands in a mouse model with coherent Raman imaging

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Sebaceous glands, which secrete sebum (a lipid-rich material) that moisturizes and protects skin, play an important role in the pathogenesis of acne vulgaris. The glands are comprised of cells known as sebocytes, which synthesize, store, and finally release sebum into hair shafts. Pharmaceutical interventions for acne improve symptoms by altering the chemical or physical nature of the glands. Interestingly, lipid-rich cells such as sebocytes have long been observed to be particularly sensitive to cold-induced injury. This cold treatment approach has recently been demonstrated to damage sebaceous glands by selectively killing subcutaneous fat cells through a process known as cryolysis. As cryolysis represents a potential therapy for acne, there is a need to understand the fundamental mechanisms that lead to improved therapy.

Coherent Raman imaging, which is an especially potent technique for visualizing lipids, has been applied to the study of cold treatment of mouse skin. Using CARS microscopy, we have non-invasively tracked and monitored the damage and subsequent recovery of individual sebaceous glands in living mice over the course of weeks following cold treatment. There are signs of both chemical and morphological alterations throughout the recovery process, including a potential reduction in sebum content in the weeks following treatment.

Lipid crystallization has been proposed as a potential mechanism that could lead to sebocyte death, but little evidence currently exists to support this hypothesis. Current studies are focused on tracking the chemical alterations underlying sebocyte cold treatment and long-term response using CARS and fluorescence microscopy along with Raman spectroscopy.

8948-33, Session 6

CARS in the single-molecule limit *(Invited Paper)*

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The Raman response of molecules provides a window for examining the vibrational motion of chemical bonds and groups. The Raman read-out is particularly interesting in the single molecule limit, where it reports on the vibrational energy shifts and phase fluctuations of the molecule as it interacts with the world around it. By using surface plasmon fields as local amplifiers of the sponatenous Raman response, it is possible to collect evolving vibrational spectra of single molecules. Although the spontaneous Raman signal provides a wealth of information about the molecule, it is incoherent and does not provide direct access to the time-evolving phase of the vibration. Coherent methods such as coherent anti-Stokes Raman scattering (CARS) offer a more direct way to interrogate vibrational phase. Yet, from an experimental point of view, driving the vibrational CARS response with surface plasmon fields has proven notoriously difficult due in part to the strong electronic four-wave mixing signals from the metal substrate, the unfavorable heat dissipation kinetics of the nano-antennas and the phase dispersion of the plasmon modes in the ensemble. In this contribution we demonstrate an experimental scheme that overcomes previous experimental limitations. We show the time-resolved, vibrational CARS response of molecules in the low copy limit, down to the single molecule limit. Our measurements, which are performed under non-electronic resonance conditions, establish that the coherent response from individual vibrational modes can be studied experimentally, opening up a new realm of molecular spectroscopic investigations.

8948-34, Session 6

Microsecond scale spectroscopic imaging by parallel detection of stimulated Raman scattering

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Raman spectroscopic imaging of highly dynamic systems was inhibited by relatively long spectral acquisition time. Recently developed multiplex coherent anti-Stokes Raman scattering (CARS) microscopy reduced the acquisition time to tens of millisecond. The CARS signal is, however, mixed with a pixel-dependent nonresonant background, which makes quantitative analysis difficult. Here, we report a novel spectroscopic imaging scheme based on parallel lock-in free detection of spectrally dispersed stimulated Raman scattering signal using a homebuilt tuned amplifier array. Our method reduced the spectral acquisition time to 30 microseconds per pixel, which is faster than multiplex CARS by three orders of magnitude. Aided by multivariate curve resolution analysis, we have monitored molecular penetration into skin tissue in situ and in real time. Fast spectroscopic imaging opens a new window for in situ analysis of target molecules in highly dynamic environment such as live cells. The reported technique also holds the potential for direct visualization of chemistry that occurs at microsecond time scale.

8948-35, Session 6

Hyperspectral SRS imaging: data analysis and applications

Dan Fu, Xiaoliang Sunney Xie, Harvard Univ. (United States)

Hyperspectral SRS imaging has become a powerful tool for analyzing complicated biological systems. The rich data obtained often confound data interpretation. We present a new data visualization method - spectral phasor - that allows a global graphic overview of the Raman spectral property of the sample. The spectral phasor method utilizes Fourier Transform to reduce three-dimensional data to two-dimension data, thus providing a convenient and robust approach towards sample segmentation based on its chemical composition. We also explore biological applications of hyperspectral SRS imaging both in the C-H and in the fingerprint region, showing its potentials and limitations in analyzing various biological samples.

8948-36, Session 6

Multimodal microscopy with high resolution spectral focusing CARS

Tommaso Baldacchini, Ruben Zadoyan, Newport Corp. (United States)

In this work we describe a device that extends capabilities of multiphoton microscopes that use dual wavelength output femtosecond laser sources to new modalities such as CARS and SRS with 15 cm-1 spectral resolution. Maximal contrast and signal is achieved when CARS is performed with spectral resolution matched with the Raman line width. Our goal in this work was to develop an approach that provides flexibility with choosing the spectral resolution. The approach is based on spectral focusing where we utilize grating based pulse stretcher in both beams. In this manner the dispersion of the stretcher can be continuously adjusted in wide range. The best spectral resolution is achieved when the slopes of chirps in both pump and Stokes beam are the same. The device is automated and any change in beam path lengths due to the stretcher or wavelength tuning is compensated by the delay line. We also incorporated into the device a computer controlled beam stabilization system that compensates the beam pointing deviation due to dispersion in the system. High level of automation and computer control allow user friendly operation. We will present CARS images of several samples that demonstrate high spectral resolution and high contrast.

8948-37, Session 7

Advances in speed, reliability, and utility of broadband CARS microscopy *(Invited Paper)*

Marcus T. Cicerone, Charles H. Camp Jr., Evangelose Gatzogiannis, Young Jong Lee, National Institute of Standards and Technology (United States)

I will report on recent advances from our lab in broadband coherent Raman imaging. We have taken advantage of unique characteristics of broadband coherent anti-Stokes Raman (BCARS) and recent advances in laser technology to significantly improve detection limits for BCARS. The new signal generation and detection schemes also provide faster and more reliable spectral retrieval without user intervention. I will argue that BCARS has now crossed the threshold between an intriguing curiosity to a tool that will be of significant utility in medicine and industry. I will also outline what I believe to be the fundamental limits to the speed and sensitivity for BCARS.

8948-38, Session 7

Fiber bundle-based endomicroscopy prototype with two collection channels for simultaneous multimodal coherent anti-Stokes Raman scattering and second-harmonic generation imaging

Zhengfan Liu, Beijing Institute of Technology (China) and Houston Methodist Research Institute (United States) and Weill Cornell Medical College (United States); Zachary A. Satira, The Methodist Hospital Research Institute (United States) and Rice Univ. (United States); Xi Wang, The Methodist Hospital Research Institute (United States) and Weill Cornell Medical College (United States); Xiaoyun Xu, Xu Chen, Kelvin K. Wong, Houston Methodist Research Institute (United States) and Weill Cornell Medical College (United States); Shufen Chen, Jianguo Xin, Beijing Institute of Technology (China); Stephen T. Wong, Houston Methodist Research Institute (United States) and Weill Cornell Medical College (United States)

Label-free multiphoton imaging is a promising modality for optical biopsy and offers new strategies for intraoperative and surgical applications. Within label-free non-linear microscopy techniques, coherent anti-Stokes Raman scattering (CARS) imaging provides strong lipid-band contrast while second harmonic generation (SHG) imaging is useful for imaging collagen and muscle fibers. A combination of the two imaging modalities could provide rich information to investigate diseases. However, as far as we know, simultaneous combination of these two imaging modalities in an endomicroscopic setting has never been reported. In this report, a fiber bundle consisted of one excitation fiber and 18 collection fibers was investigated in our endomicroscopy prototype. The 18 collection fibers were divided into two collection channels with 9 fibers in each channel. These two channels could be used together as one channel for efficient signal collection or used separately for simplifying signal detection. Differences of collection pattern of these two channels were investigated. Collection difference of central excitation fiber and surrounding 18 fibers was also investigated, which reveals potential ability of this system to measure forward to backward (F/B) ratio in SHG imaging. CARS imaging of mouse adipocyte and SHG imaging of mouse tail tendon were performed to demonstrate tissue imaging ability of this system. Simultaneous CARS and SHG imaging ability of this system was demonstrated in mouse tail imaging. This fiber bundle based endomicroscopy imaging prototype, offers a promising solution for efficient fiber-based CARS and SHG multimodal endomicroscopy for minimally invasive intraoperative applications.

8948-39, Session 7

High-performance fiber parametric oscillator for coherent Raman microscopy

Erin S. Lamb, Simon Lefrancois, Cornell Univ. (United States); Minbiao Ji, Harvard Univ. (United States); William J. Wadsworth, Univ. of Bath (United Kingdom); X. Sunney Xie, Harvard Univ. (United States); Frank W. Wise, Cornell Univ. (United States)

A compact, alignment-free, and inexpensive fiber source for coherent Raman spectroscopy (CRS) would benefit the field considerably. We present a fiber optical parametric oscillator that matches the pulse parameters of solid-state lasers used in CRS and offers the best performance from a fiber source to date. Pumping the oscillator with pulses from a 1 ?m fiber laser, we achieve widely spaced, narrowband pulses suitable for coherent anti-Stokes Raman scattering microscopy. Transform limited, 2 picosecond signal pulses are generated through the use of normal dispersion four wave mixing in photonic crystal fiber, and can be tuned from 779-808 nm, which corresponds to frequency shifts of 2740 cm-1 to 3150 cm-1 from the pump, limited by the tuning range of the seed laser. The average signal power can reach 180 mW (pulse energies up to 4 nJ). The long-wavelength idler field is resonant in the oscillator, and the use of a narrow bandpass filter in the feedback loop is critical for stable operation, as seen in both simulation and experiment. This source has much lower intensity fluctuations than parametric amplifiers based on the same frequency conversion process. We present high quality images of mouse tissues taken with this source that exhibit an outstanding signal to noise ratio at top imaging speeds.

8948-40, Session 7

Tunable dual-wavelength 2-picosecond light source for coherent Raman scattering microscopy

Ingo Rimke, APE GmbH (Germany); Gregor F. M. Hehl, Univ. Stuttgart (Germany); Marcus Beutler, Peter Volz, APE GmbH (Germany); Andreas Volkmer, Univ. Stuttgart (Germany); Edlef Büttner, APE GmbH (Germany)

In narrow-bandwidth coherent Raman scattering (CRS) microscopy, efficient signal generation is accomplished with two-color laser sources providing synchronized picosecond pulses whose frequency difference and spectral widths match the molecular Raman frequency and bandwidth, respectively. With vibrational bandwidths of typically 10 cm-1, the optimum laser pulse durations thus correspond to about 2 ps.

Here, we present a new light source consisting of an amplified Yb-fiber oscillator providing 2-ps pulses at 1030 nm and a synchronously green-pumped optical parametric oscillator (OPO). The OPO slightly shortens the pulses to <2 ps while maintaining a bandwidth of 10 cm-1. Output power levels of 1 W in both the 1030-nm and the OPO-branch with continuously tunable frequency differences between the two beams over a broad range from 700 to 4500 cm-1 are achieved. In addition to CARS microscopy, this light source allows for SRS imaging via an integrated electro-optical modulation of the 1030-nm beam at 20 MHz with a depth of >95%, locked to the laser repetition rate of 80 MHz. The OPO noise at 20 MHz was found to be only a factor of 2 above the combined detector and laser noise using a conventional Nd:YVO pump source. This represents a significant reduction in laser noise when compared to other fiber-based laser sources previously proposed for SRS microscopy.

Applications of SRS imaging with this new light source will be demonstrated and compared with a 7-ps solid-state Nd:YVO laser pumped OPO source with respect to the achieved SRS signal enhancement and image contrast.

8948-41, Session 7

Fourth-order coherent Raman microspectroscopy for detection of material symmetry

Mamoru Hashimoto, Hiroto Kanoh, Hirohiko Niioka, Tsutomu Araki, Osaka Univ. (Japan)

Recently, coherent Raman microscopy has become an attractive visualizing tool because of chemical imaging using molecular vibrations. In order to add another information to molecular vibrations, we apply fourth order coherent Raman spectroscopy for imaging. Fourth order coherent Raman process is anti-Stokes hyper-Raman scattering of the coherently excited molecular vibration with Raman process. It is known that even order nonlinear process is forbidden for a centrosymmetric material. Therefore, the information of material symmetry can be added to the information of molecular vibrations with fourth order coherent Raman.

We have developed a multiplex fourth order coherent Raman microspectroscope. The system is similar to the multiplex CARS microscope using supercontinuum light generated with a photonic crystal fiber, and the difference with CARS microscopy is the observed Raman shift region. Hence, we can obtain CARS and fourth order coherent Raman spectral images by changing optical filters and the observed region of spectrometer. Hyper Raman spectrum and second harmonic generation are also observable.

We have applied the developed system to the imaging of recrystallized organic molecule in saturated solution. The image of recrystallized molecule was visualized by fourth order coherent Raman without any interference from the saturated solution because the fourth order coherent Raman is forbidden for randomly oriented bulk liquids.

8948-42, Session 7

Deep-tissue chemical imaging using CARS microspectroscopy

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CARS microspectroscopy is an established method to acquire chemically specific information from a microscopic volume. Under certain conditions, CARS microspectroscopy can provide the same information as Raman spectroscopy, but much faster. Over the past years, multiphoton imaging modalities made significant strides to extend the depth of imaging to several millimeters inside the tissue [1]. CARS microspectroscopy, being a nonlinear optical technique, should provide the same advantage over conventional Raman imaging for deep (>1-mm) tissue imaging. Most of the present deep tissue chemically-specific imaging is based on surface offset Raman spectroscopy [2] and Raman tomography [3], which have limited ability to spatially resolve objects hidden below a scattering layer.

In our approach, we followed the guidelines of Denk et al [4], i.e. used higher energy per pulse, longer excitation wavelength and better collection efficiency of the signal, to achieve good spatial resolution imaging through mm-thick scattering medium [5-7]. Recently, guided by Monte Carlo simulations, we improved the geometry of excitation and, using novel imaging optics and spectral analysis software, substantially increased the efficiency of light collection, which allowed achieving even deeper penetration depth without sacrificing the imaging time for hyperspectral chemical analysis.

In the presentation, we will review the prior approaches, outline the theoretical foundation of the new apparatus, illustrate the advances on this new approach using well-established experimental model systems, outline the potential applications for imaging both static and dynamic systems in vivo, and evaluate the ultimate limits of the imaging depth attainable by means of nonlinear Raman microspectroscopy.

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8948-43, Session 7

Multiphoton imaging of biological samples during freezing and heating

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Temperature-induced effects in biological samples have great commercial (e.g. for cryopreservation) and scientific applications in many areas. However, the possibilities to online monitor samples with subcellular resolution during freezing and storing processes remain limited. We apply multiphoton microscopic imaging based on fluorescence to observe freezing and heating effects in plant- and animal cell samples. Temperature-dependent fluorescence lifetimes (FLIM) are in addition measured and analyzed using the so-called phasor method. The experimental setup consists of a multiphoton imaging system and a heat and cooling stage which allows for precise temperature control from 77 K to 873 K. To demonstrate the imaging capabilities (Chinese hamster ovary) CHO cell samples were imaged during freezing-thawing cycles between room temperature and -80°C. Cooling resulted in an increase of both the intracellular fluorescence intensity and lifetime as well as in morphological changes. Furthermore, the temperature dependence of the fluorescence intensity and lifetime from a sample of the botanical model plant Arabidopsis Thaliana was investigated. The results also showed morphological changes and a shift of the main fluorescence intensity from chlorophyll to cell membrane fluorescence with decreasing temperature. The measurements illustrate the usefulness of multiphoton imaging and CARS to investigate freezing and thawing effects in animal and plant cells in general and in particular at temperatures used for cryopreservation.

8948-44, Session 7

Imaging drug diffusion in living skin equivalent membranes using hyperspectral stimulated Raman scattering microscopy

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Efficient drug delivery to the skin is essential for the treatment of major dermatologic diseases, such as eczema and psoriasis. However, many compounds penetrate the skin barrier poorly and require optimized formulations to ensure their bioavailability. Recently, the use of living skin equivalents (LSE) has become popular for transdermal permeation. LSEs offer several advantages over real skin for studying dermal permeation; they eliminate animal experimentation; they use human skin cells (which provide skin properties similar to those found in native human skin); and most importantly they contain all of the lipids found in the native human skin.

The delivery of fatty acids into the skin is of particular interest to the pharmaceutical industry and mapping the diffusion of these compounds at the cellular level (in LSEs) provides vital information that can aid rational engineering of compounds with enhanced permeation. However, conventional fluorescence microscopy methods are not desirable since conjugating these low molecular weight molecules with fluorphores is known to perturb their transport kinetics.

Stimulated Raman scattering (SRS) microscopy uniquely provides label-free, nondestructive, three-dimensional images with high spatiotemporal resolution and is an ideal tool for this application. However, distinguishing between the applied fatty acids and intrinsic skin lipids is not possible using a single Raman mode. We demonstrate that by acquiring a series of SRS images over the full range of the CH region it is possible to monitor the penetration of the applied formulation within LSEs to reveal novel features of (trans)dermal drug delivery in the living tissue environment.

8948-45, Session 8

Recording transient fluorescence lifetime effects by TCSPC FLIM *(Invited Paper)*

Wolfgang Becker, Vladislav Shcheslavskiy, Becker & Hickl GmbH (Germany); Samuel Frere, Inna Slutsky, Tel Aviv Univ. (Israel)

We present a technique that records transient effects in the fluorescence lifetime of a sample with spatial resolution in one or two dimensions. The technique is based on a multi-dimesnional photon counting process that builds up a photon distribution over the arrival times of the photons after the excitation pulses, the time after a stimulation of the sample, and one or two spatial coordinates. The maximum resolution at which lifetime changes can be resolved is on the order of a few milliseconds. We demonstrate the technique for recording chlorophyll transients in live plants and for Ca++ imaging in cultured neurons.

8948-46, Session 8

Fluorescence-guided tumour diagnosis, cell metabolism, and multispectral FLIM *(Invited Paper)*

Angelika C. Rueck, Carmen Hauser, Univ. Ulm (Germany); Adrian Ruehm, Max-Planck Institut für Intelligente Systeme (Germany); Herbert Stepp, Univ. Hospital Munich (Germany); S. Kalinina, Univ. Ulm (Germany)

Fluorescence guided diagnosis of tumour tissue is in many cases insufficient, because false positive results are interfering with the outcome. Discrimination between tumour and inflammation could be therefore difficult. Improvement of fluorescence diagnosis through observation of cell metabolism could be the solution, which needs a detailed understanding of the origin of autofluorescence. However, a complex combination of fluorophores give rise to the emission signal. Also in PDD (photodynamic diagnosis) different photosensitizer metabolites contribute to the fluorescence signal. Therefore, the fluorescence decay in many cases does not show a simple monoexponential profile. In those cases a considerable improvement could be achieved when time-resolved and spectral-resolved techniques are simultaneously incorporated [1].

The discussion will focus on the detection of NADH, FAD and 5-ALA induced porphyrins. With respect to NADH and FAD the discrimination between protein bound and free coenzyme was investigated with multispectral FLIM in normal oral keratinocytes and squamous carcinoma cells (SCC) from different origin. FLIM of free and protein bound NADH of OKF6, SCC25 and SCC4 cells is demonstrated in figure 1. The redox ratio, which can be correlated with the fluorescence lifetimes of NADH and FAD changed depending on the state of the cells [2].

Most of the investigations were done in monolayer cell cultures. However, in order to get information from a more realistic in vivo situation additionally the chorioallantoismembrane (CAM) of fertilized eggs was used where tumour cells or biopsies were allowed to grow. The results of theses measurements will be discussed as well.

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8948-47, Session 8

High-sensitivity single molecule fluorescence detection using scanning single-molecule counting

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A new, simple technique for single molecule fluorescence detection has been developed and detection of 100 aM fluorophores has been demonstrated. The technique, similarly to Fluorescence Correlation Spectroscopy (FCS), Photon Counting Histogram (PCH) and Fluorescence Intensity Distribution Analysis (FIDA), uses a confocal optical system, but differs in that it detects individual molecules crossing the inside of a scanning confocal volume without using statistical techniques as applied in FCS or similar methods. The scanning speed of the confocal volume is higher than the Brownian motion speed of the light emitting molecules. Thus, the time evolution of the light intensity data reflects the confocal volume intensity profile, which clearly shows the crossing of single molecules. The proposed technique is based on the simple principle of counting molecules one by one using a scanning confocal volume, and is hereafter referred to as Scanning Single-Molecule Counting (SSMC). The total scanned volume of a solution estimated from the confocal optical system, enables the concentration or number density of molecules to be obtained. The measurement of the polarization of individual molecules was attempted, and it was found that one molecule can provide information on the rotational diffusion of the molecule. Fast overlapping scans detected multiple signals from a single molecule at almost the same position, which allowed the translational diffusion constant of the molecule to be estimated.

8948-48, Session 8

FAK-cTERM/?B-crystallin interaction by Förster resonance energy transfer (FRET)

Aline M. dos Santos, Ctr. Nacional de Pesquisa em Energia e Materiais (Brazil); André A. de Thomaz, Univ. Estadual de Campinas (Brazil); Michelle B. M. Pereira, Ctr. Nacional de Pesquisa em Energia e Materiais (Brazil); Diogo B. Almeida, Vitor B. Pelegati, Mariana O. Baratti, Carlos L. Cesar, Univ. Estadual de Campinas (Brazil); Kleber G. Franchini, Ctr. Nacional de Pesquisa em Energia e Materiais (Brazil) and Univ. Estadual de Campinas (Brazil)

FRET allows a direct access of protein interaction partners in their natural environment inside the cell. Here, we report a direct interaction of Focal adhesion kinase (FAK) with the protein chaperone ?B-crystallin by FRET. In this study, the cyan fluorescent protein coupled to ?Bcrystallin (CFP-CryAB) served as the FRET donor, while the yellow fluorescent protein coupled to the C-terminus region of FAK (YFP-cTERM) served as the FRET acceptor. FRET, which was measured using fluorescence lifetime imaging microscopy (FLIM), occur if the fluorophores are located within 2-10 nm of one another and are oriented favorably with respect to each other. Plasmid vectors encoding unfused CFP and YFP were co-transfected in HEK cells as negative controls. Despite the co-localization of the donor-acceptor fluorophores in the confocal images, the fluorescent lifetime weighted mean component (?m) of the donor was 2.5 ns, indicating the absence of FRET. A highly efficient positive FRET control was obtained with the CFP fluorophore coupled directly to YFP through a 15 amino-acid-long linker. In this case, the lifetime of CFP decreased to 1 ns, indicating the proximity of CFP to YFP. The ?m of CFP-CryAB co-expressed with YFP was 2.4 ns while the co-expression of the pair CFP-CryAB and YFP-cTERM decreased the donor lifetime to 1.8 ns. This decreased in the lifetime of the CFP-CryAB confirmed a positive FRET between CFP-CryAB and YFP-cTERM. Thus, the FRET assay was efficiently utilized to demonstrate a direct interaction between FAK cTERM and the chaperone ?B-crystallin in living cells.

8948-49, Session 8

Decay pattern matching analysis for multi-label FLIM

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In order to understand the function and interaction of cellular compartements ist is often required to stain different cellular proteins with fluorophor labeled probes, e.g. dye labeled antibodies. In conventional fluorescence microscopy different fluorophores are separated due their spectral properties, i.e. by splitting the fluorescence signal on spectrally different channels.

In general suitable fluorophores can also be separated due to differences in their fluorescence lifetime, but a quantitative analysis of multicomponent FLIM data is time consuming and prone to errors if the lifetime decay patterns are complex (multi-exponential). We present a novel alternative to multi-exponential decay fitting and also to the phasor analysis approach. We describe the complex single pixel decay with a linear combination of reference decays which can be highly multi-exponential and do not have be described itself mathematically. The low number of fitting parameters result in a faster analysis and a better signal to noise ratio in the analysed images.

In addition, this decay pattern matching approach can also be expanded to cope simultaneously with decay and spectral data which are recorded with a 32 channel TCSPC-PMT. The combination of multicolour pulsed interleaved excitation with spectrally- and time-resolved single photon detection is the ultimate method for multi-label experiments in complex, like cellular, environments. We will show latest results of separating quantitatively up to 3 dyes just based on their fluorescence decays and more then 5 dyes be taking also the spectral information into account.

8948-50, Session 8

Quantification of single FRET-labeled DNA and FoF1-ATP synthase by confocal FLIM-FRET microscopy and FCS/FCCS

Nawid Zarrabi, Marc Renz, Friedrich-Schiller-Univ. Jena (Germany); Bertram Su, Wolfgang Becker, Becker & Hickl GmbH (Germany); Michael Börsch, Friedrich-Schiller-Univ. Jena (Germany)

Analysis of oligomerization or conformational changes of protein machines in living cells can be achieved by applying Förster resonance energy transfer (FRET) between two attached fluorescent markers. FRET measures internal distances between the FRET donor and the acceptor dye in the range of 2 nm to 10 nm. Therefore, fluorescence intensities or donor fluorophore lifetimes (FLIM) are analyzed. However, unraveling the exact composition of multiple FRET-labeled proteins and other fluorecent molecules in a single pixel of a confocal image remains a challenge. Detecting only a single molecule at a time is used to overcome the problem of averaging. Here we show how single molecules can be analyzed quantitatively by confocal time-resolved FLIM-FRET in combination with fluorescence correlation (FCS) and cross-correlation spectroscopy (FCCS). A new hybrid algorithm improved data analysis times by a factor of 15. The one-FRET level DNA sample is compared to the rotary motor FoF1-ATP synthase with three fast interchanging FRET levels, and the potential for quantitative description of fluorescent components in a single pixel is demonstrated.

8948-51, Session 8

Cryogenic confocal microscopy platform integrated with fluorescent lifetime imaging and spectroscopy

Diogo B. Almeida, André A. de Thomaz, Vitor B. Pelegati, Hernandes F. Carvalho, Carlos L. Cesar, Univ. Estadual de Campinas (Brazil)

Nonlinear optical microscopy (NLOM) has spread in research groups around the world to perform optical characterization of samples resolved in space, time and spectrally. A complete physical and chemical property characterization of materials require samples cooled at cryogenic temperatures. However, high resolution confocal laser scanning microscopy and cryogenics are not easily coupled together due to vacuum and long working distance requirements. In this paper we demonstrated a cryogenic NLOM platform capable to perform several confocal microscopies and spectroscopies. We have demonstrated the use of such system to observe Two-Photon Excited Fluorescence (TPEF), Fluorescence Lifetime Imaging (FLIM), Second Harmonic Generation (SHG) microscopies, as well as fluorescence, 1 and 2 photons photoluminescence excitation (PLE), Raman and other nonlinear optical spectroscopy, all in the range of 10-300K. The assembly was achieved through the adaptation of a small cryostat into a commercial Zeiss LSM 780 NLO confocal microscope. A tunable fs laser was used as the source of NLO microscopies, as well as the source for 2-photon PLE. A 30 cm spectrophotometer confocally aligned have been used to acquire 1-2 photons fluorescence, PLE, Raman, SHG spectra in any region of interest ROI of the sample. The whole system spectral response was calibrated

with a black body radiator to obtain quantitative results. We show the quality and functionality of this setup with images and spectroscopy results for colloidal quantum dots samples as a function of temperature. We, therefore, demonstrated an integrated NLO platform capable of optical characterization with spatial, temporal, spectral and thermal resolution.

8948-52, Session 9

Fluorescence lifetime imaging of pluripotent cells *(Invited Paper)*

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The multiphoton FLIM tomograph MPTflex with its flexible scan head, articulated arm, and the tunable femtosecond laser source was employed to study cell monolayers and 3D cell clusters. FLIM was performed with 200 ps temporal resolution and submicron spatial resolution using time-correlated single photon counting. The autofluorescence based on NAD(P)H and flavins/flavoproteins has been measured on stem cells and iPS cells.

8948-53, Session 9

Regulatory conformational changes of the epsilon-subunit in single FRET-labeled FoF1-ATP synthase *(Invited Paper)*

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Subunit epsilon is an intrinsic regulator of the FoF1-ATP synthase, the ubiquitous membrane-embedded enzyme that utilizes a proton motive force to synthesizes adenosine triphosphate (ATP). In the Escherichia coli enzyme the C-terminal domain of subunit epsilon can extend into the central cavity formed by the stator subunits alpha and beta, to block the rotation of the central gamma subunit and, thereby, to prevent wasteful ATP hydrolysis. Here we aim to determine the conditions under which subunit epsilon can inhibit the FoF1-ATP synthase in vivo. We use the fluorescent dye FlAsH to specifically label subunit epsilon via a tetra-cysteine motive within its C-terminal domain. When FlAsH changes its spatial orientation the regulatory conformational change of subunit epsilon is monitored by single-molecule anisotropy microscopy.

8948-54, Session 9

Megapixel FLIM by multi-dimensional TCSPC

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Multi-dimensional TCSPC is based on the excitation of a sample by a high-repetition rate laser and the detection of single photons of the fluorescence signal. Each photon is characterised by its time in the laser period, its wavelength, and the coordinates in the scanning area. The recording process builds up a photon distribution over these parameters. The result can be interpreted as an array of pixels, each containing a full fluorescence decay curve, or even several fluorescence decay curves for different wavelength. Therefore, FLIM data require much more memory space than steady-state images. Memory space was a problem for Windows 32-bit software: The size of the images was limited by the addressable computer memory rather than by the capabilities of the TCSPC module. We present a new 64-bit FLIM acquisition software that takes full advantage of the capabilities of Windows 64 bit. As a result, FLIM data can be recorded with unprecedented numbers of pixels and time channels. We demonstrate the performance for applications that require imaging of a large number of cells in a single field of view, for parallel-channel FLIM in several wavelength channels, and for multi-wavelength FLIM.

8948-55, Session 9

Fluorescence correlation spectroscopy measurement of the hydrodynamic radius of colloidal quantum dots excluding blinking

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The most used techniques to measure Quantum Dots (QDs) sizes, such as Transmission Electron Microscopy (TEM), or X-Ray Diffraction, usually observe the nuclei distance and not the electron confinement distance. This could mean a big difference in 6 to 10 atomic layers QDs. Besides, TEM sampling depends on the observer and the sample preparation can change the QD size. X-ray diffraction, usually SAXS, sampling is very good but the technique has its own difficulties and artifacts. Moreover, the processed samples are not in their colloidal form eliminating any possibility of observation of surface interaction with the environment.

Fluorescence Correlation Spectroscopy (FCS) can observe single particles, over a great number of particles, in colloidal environment, which makes it ideal to study the hydrodynamic radius (HR) of colloidal QDs. The HR also allows us to observe also QD with cap layers. However, blinking effects can change completely FCS curves providing wrong measurements. Most reports on this subject used the argument that blinking is not important at low power. However we observed blinking in our experiment even at lowest power. The correct diffusion time, and consequently, the correct HR is obtained only in the absence of blinking, for laser power tending to zero. We measured the HR over a series of laser powers and observed an exponential behavior form which we can extrapolate the zero power limit and obtained the blinking free QD HR. Our results allowed us to compare different confinement energy theoretical models and show discrepancies between them.

8948-56, Session 9

An automated image processing routine for segmentation of cell cytoplasms in high-resolution autofluorescence images

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The ability of clinically used biomarkers, such as estrogen receptor (ER) and HER2, to predict therapy response is limited due to patients that present de novo resistance. We investigate the use of multiphoton fluorescence lifetime imaging of the metabolic coenzymes NADH and FAD, for characterization of breast cancer sub-types and prediction of therapeutic efficacy. Fresh clinical breast cancer tissue samples are obtained from resection surgeries and imaged immediately. In addition, the clinical tissues are dissociated into organoids which are cultured in a collagen matrix, exposed to traditional and experimental breast cancer drugs, and imaged at 24, 48 and 72 hr post drug exposure to evaluate cellular drug-induced responses. In intact tissues, NADH and FAD mean lifetime values correlate with estrogen receptor and HER2 expression, which are known modulators of cellular metabolism. Significant reductions ($p<0.05$) in optical metabolic endpoints are detected in organoid cultures derived from estrogen receptor positive primary human tumors treated with tamoxifen and combination therapies. Likewise, significant reductions ($p<0.05$) in mean NADH lifetimes are observed in trastuzumab treated HER2+ organoid cultures, while no significant differences ($p>0.05$) in optical endpoints are observed in triple negative

breast cancer derived organoids treated with targeted therapies. These changes in optical endpoints measured in organoid cultures of primary human breast tissues agree with the expected tumor response to drug treatments, based on immunohistochemistry and gold standards of receptor status. Together, this data suggests that fluorescence endpoints of metabolism are powerful biomarkers for classification of breast tumors and predicting therapeutic efficacy.

8948-57, Session 9

A molecular imaging analysis of Cx43 association with Cdo during skeletal myoblast differentiation

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Cdo is a multifunctional cell surface protein with immunoglobulin and fibronectin III repeats in its ectodomain, and a long intracellular region exerts its promyogenic action functioning as a component of the cadherin-complexes. This molecule binds to N-cadherin and upon cell cell-cell adhesion, mimicked by N-Cadherin ligation, undergoes activation via interaction with signaling and adaptor proteins, including p38MAPK, thereby initiating intracellular signal transduction cascades which through phosphorylation of substrates, stimulate MyoD-dependent, muscle-specific gene expression. Its critical role during myogenesis is also revealed by the results showing that mice lacking Cdo exhibit delayed skeletal myogenesis, and Cdo?/? primary myoblasts have defects in myoblast differentiation. connexin43 (Cx43), the main connexin isoform expressed in skeletal myoblasts, besides forming intercellular channels which regulate the trafficking and functional integration among the adjacent myoblasts, serves a gap-junction independent pro-myogenic function.

On the basis of all these considerations, in the present study we searched for a possible functional interaction between Cx43 and Cdo as a preliminary attempt to expand our knowledge on the biological functions of Cx43 in skeletal myogenesis. By combining different optical microscopic techniques, ranging from confocal immunofluorescence to hyperspectral FLIM-FRET, we showed that Cx43 and Cdo displayed the same spatiotemporal expression pattern and physically interact to form dynamic complexes in C2C12 cells during myogenesis, offering clues for considering this interaction a structural basis of the channel-independent function of Cx43.

8948-58, Session 10

Expanding multiphoton excitation/absorption approaches into superresolution methods *(Invited Paper)*

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It is well known and established that, for the most popular imaging mode in optical microscopy, i.e. fluorescence, the diffraction barrier does no longer provide an unsurpassable limitation for resolution and localization accuracy. Furthermore, the terms “super resolution” and “optical nanoscopy”, coined earlier, have been implemented in real far field optical microscopes, nowadays available for everyone to use without extreme complexity. Here, we will discuss targeted and stochastic readout methods expanding multi-photon excitation(MPE)/absoprtion, in terms of resolution and localization precision accuracy. Individual molecule localization (IML) implemented within selective plane illumination microscopy (SPIM) will be addressed towards 3D super resolution imaging in thick biological samples including non linear photo-activation. MPE-STED microscopy will be discussed reporting about the utilization of a single wavelength (SW) both for MPE and fluorescence depletion. A variety of architectures will be outlined and further variations on the super resolution theme addressed, including coupling with atomic force microscopy (AFM) and nanoscale lithography.

8948-59, Session 10

Origins of contrast in multiphoton microscopy imaging of melanoma *(Invited Paper)*

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We employed a clinical multiphoton microscope (MPTflex, JenLab, Germany) to image in vivo and non-invasively melanocytic nevi at three different stages: common nevi without dysplastic changes, dysplastic nevi with structural and architectural atypia, and melanoma. Two-photon excited fluorescence (TPEF) was used for visualizing endogenous fluorophores such as NADH/FAD, keratin, melanin in the epidermal cells and elastin fibers in the dermis. Collagen fibers were imaged by second harmonic generation (SHG). We analyzed the multiphoton microscopy (MPM) images corresponding to 15 lesions (5 in each group) both qualitatively and quantitatively. For the qualitative analysis, we identified the morphological features characteristic to each group. Morphological changes such as cytological atypia and lentiginous hyperplasia correlate with the TPEF signal. Likewise, morphological changes at the epidermal-dermal junction such as appearance of nests of nevus cells on the sides of the rete ridges or disruption of the junction correlate with variations in the SHG signal. We defined a numerical “multiphoton melanoma index (MMI)” based on quantitative TPEF, SHG, and density of melanocytic dendrites in the upper epidermal layers. These parameters were derived from 3D image analysis. We show that the MMI scores corresponding to each group are significantly different from the scores in the other two groups. The results of this study provide a set of morphological MPM features typical to common, dysplastic nevi, and melanoma, along with an algorithm based on quantitative measurements, which shows great potential to discriminate these groups of melanocytic lesions.

8948-60, Session 10

Modulation of the pupil function of microscope a objective lens for multifocal multiphoton microscopy with a spatial light modulator

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We propose a method of high precision modulation of the complex pupil function of microscope objective lens to improve the performance of Multifocal Multi-photon Microscopy (MMM). To modulate the pupil function, we adopt a phase modulation type of spatial light modulator (SLM) to place it at the conjugate position of the objective lens. The SLM can generate arbitrary number of multiple spots for exciting the multiple fluorescence spots (MFS) at desired positions and intensities by applying an appropriate computer generated hologram (CGH). Such flexibility

allows us to control the MFS according to the photobleaching level of a fluorescent protein and phototoxicity of a specimen. However, when a large number of excitation spots are generated, the intensity distribution of the MFS is significantly different from the designed one due to misalignment of the optical setup and the SLM characteristics. As a result, image of specimen by using the laser scanning for MFS has block noise segment because the SLM could not generate the uniform MFS.

To improve the intensity distribution of MFS, we adaptively redesigned CGH based on the observed MFS. We experimentally demonstrate an improvement of the uniformity of the 10?10 grid of MFS by using a dye solution with consistent uniformity. Because of its simplicity of the proposed method, it can be applied for calibration of the MMM before observation of living tissue. After calibration of MMM, we performed laser scanning with two-photon excitation to observe a real specimen without block noise segment.

8948-61, Session 10

A novel clinical multimodal multiphoton tomograph for AF, SHG, CARS, and FLIM

Karsten König, Univ. des Saarlandes (Germany); Martin Weinigel, JenLab GmbH (Germany); Hans Georg Breunig, Univ. des Saarlandes (Germany)

Clinical multiphoton tomography is used for non-invasive high resolution tissue imaging in hospitals, in the cosmetic and pharmaceutical industry as well as in small animal research. Here we present a novel flexible multimodal femtosecond laser tomograph with a flexible optomechanical arm and a compact 360° tunable scan/detection head. Two near infrared laser beams are employed to realize nonlinear autofluorescence (AF), second harmonic generation (SHG), and Coherent AntiStokes Raman Spectroscopy (CARS) with a photonic crystal fiber. Clinical data are presented.

8948-62, Session 10

Two-photon two-color excited fluorescence spectroscopy in flurophores

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We present the results of theoretical and experimental studies of a new technique for investigation of biomolecular dynamics: polarized fluorescence in flurophores excited by two-photon two-color (2P2C) femtosecond laser pulses. Quantum mechanical expressions describing the fluorescence polarization have been derived under the condition of isotropic rotation diffusion and valid for arbitrary polarization of each of the three photons involved in the photoprocess. The experiment has been carried out on p-terphenyl and 2-methyl-5-t-butyl-p-quaterphenyl (DMQ) dissolved in cyclohexane/paraffin. The experiment was performed using the 2C2P excitation scheme utilizing simultaneous absorption of two femtosecond laser pulses in the 400-440 and in 800-880 nm spectral range. Using different combinations of the photon polarizations we extracted seven time-dependent molecular parameters from experiment. The analysis of the obtained experimental data was based on ab initio calculations and gave information on two-photon excitation channels and interaction of flurophores with surrounding solute molecules. The new technique developed will be used for determining bioinformation from fluorophores embedded in biological molecules.

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8948-63, Session 11

Enhancing stimulated emission-based fluorescence detection with interferometric setup *(Invited Paper)*

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Stimulated emission, being spatially coherent, supports unattenuated fluorescence detection at extended distance with low NA optics. We have demonstrated stimulated emission (SE) imaging in a long-working distance configuration. Additionally, the corresponding fluorescence lifetime imaging is realized by electronically controlling the time delay between the excitation and the SE pulses in the nanosecond ranges through pump-probe configuration.

However, the sensitivity of SE based fluorescence detection is usually limited by the dynamic range and saturation of photodetectors. We are showing that interferometric setup can greatly enhance the detection sensitivity by reducing the DC level of the stimulation beam with destructive interference. The setup also allows optical coherence tomographic sectioning with the stimulated emission beam, reaching a resolution of approximately 25 microns that is dependent on the corresponding spectral width. The results show that there are many interesting possibilities by combining interferometric techniques with stimulated emission based fluorescence detection.

8948-64, Session 11

Nonlinear deep-UV excitation microscopy for multicolor fluorescent protein imaging with high spatial resolution

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Fluorescent proteins have become a key element for the visualization of intracellular structures and their activities under optical microscopes. Simultaneous observation of fluorescent proteins with different emission wavelengths allows us to obtain information of colocalization of different proteins and interactions of proteins with intracellular structures, which provides direct evidences of cooperative activities of molecules. In this paper, we propose a use of two-photon excitation at visible wavelength to simultaneously excite fluorescent proteins with different emission wavelengths. We have found that the irradiation of pulsed laser light at around 520 nm can excite fluorescence of Sirius, mseCFP, mTFP1, and EGFP with light absorption at the deep-UV (DUV) region. By using this technique, we have performed the simultaneous multi-color observation of HeLa cell expressing, Sirius, mseCFP, mTFP1, and EGFP at the mitochondria, nucleosome, Golgi apparatus, and nucleoli. This excitation scheme provides the spatial resolution equivalent to that with the DUV excitation, which brings about the spatial resolution of 100nm and 275 nm for lateral and axial directions. We also confirmed that the drastic improvement of the depth discrimination by comparing xz cross-section images of the intranuclear structure with the nonlinear-DUV and the single-photon excitation. We also obtained the excitation spectra of the fluorescent proteins and found that the efficiency of excitation photon to fluorescent photon conversion is similar under the nonlinear-DUV excitation at 525 nm. The comparison of the emission spectra under the nonlinear-DUV and the single-photon excitation indicates that

nonlinear-DUV excitation exhibits the photon emission through a different photophysical pathway from the single photon excitation with DUV light.

8948-65, Session 11

Aperture design in focal modulation microscopy to improve modulation depth

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Although confocal microscopy (CM) has been successfully used in biological imaging, it is less effective in probing deep inside highly scattering medium due to multiple scattering photons leaking through the confocal pinhole. Combining CM with coherence gating mechanism, focal modulation microscopy (FMM) was recently developed as a novel method for in-vivo imaging of thick biological tissues. FMM introduces a spatiotemporal phase modulator (STPM) into the illumination beam path to modulate part of the beam, which results in temporal oscillation of the interference pattern in the focal volume; whereas no oscillatory emission occurs in the out-of-focus region because of spatial separation between the modulated and unmodulated beams. By retrieving the oscillatory emission light to make FMM images, the background signal in the out-of-focus region can be effectively rejected. It has been demonstrated theoretically and experimentally that FMM can probe significantly deeper than CM, especially when the imaging performance is restricted by strong multiple scattering. Modulation depth is the most important parameter in designing STPM in FMM because it determines the signal-to-noise ratio and the efficiency of FMM signal generation. Besides penetration depth improvement, large modulation depth also reduces the threshold of excitation power, which is important for avoiding photobleaching. Here, we employ the concept of pupil-moment to optimize STPM design to increase modulation depth. This method is proven effective for high numerical aperture imaging using vector diffraction theory for both linearly and radially polarized incident beams. Several STPM designs with simple configurations and large modulation depth are proposed and demonstrated.

8948-66, Session 11

An efficient method to study electric field distortions of a tightly focused beam in a medium with microscopic scattering particles

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One of the main limitations of high-resolution optical microscopy in thick tissues is compromised image resolution and depth due to distortion of the amplitude and phase of the focal volume due to light scattering. Recent empirical methods to mitigate scattering have not provided the fundamental insights needed to improve our understanding of the relationships between cell and tissue morphology, composition, relative location and focal field distortion. An efficient model capable of analyzing the effects of scattering on the focal volume would not only advance our understanding of the focal field distortion, but also provide important clues towards mitigating the negative impacts of light scattering. We introduce a computationally efficient Huygens-Fresnel Ray based Electric Field Superposition (HF-REFS) method that can determine the focal electric field distortion by a fixed configuration of scatterers. The results show a good agreement with the results from the Finite Difference Time Domain (FDTD) solution that typically needs enormous computational resources. Current implementation of the HF-REFS method is 2-4 orders of magnitude faster than the FDTD method and does not require high performance computer systems to run simulations. This method that can provide quick snap shot of the electric fields in the region of interest provides an opportunity to quickly find the link between scatterer characteristics and its effect on amplitude and phase. We apply this method to study how size, location and number of spherical scatterers affect the displacement of the nominal focal position, and amplitude attenuation, and phase distortion relative to a non-scattering medium.

8948-67, Session 11

Monitoring of cell metabolism and lipid production in 3D engineered human adipose tissues using label-free multiphoton microscopy

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Non-linear optical microscopy methods can be used to assess dynamically over time multiple functional properties of engineered tissues during development. In this study we performed combined third-harmonic generation (THG) and two-photon excited fluorescence (TPEF) imaging measurements to identify quantitative biomarkers of adipogenic stem cell differentiation and metabolic state, respectively. Specifically, we imaged repeatedly over nine weeks silk scaffolds embedded with human mesenchymal stem cells and exposed to either propagation (PM) or adipogenic differentiation media (AM). THG was employed to visualize the formation of lipid droplets. TPEF was used to assess the metabolic state of the cells through the redox ratio defined based on the endogenous FAD and NADH fluorescence, as NADH/(FAD+NADH). The redox ratio of cells in the AM scaffold was significantly lower than that in the PM scaffold during week 5 and 9, and correlated with significant increases in lipid-to-cell volume ratio, and number and size of lipid droplets in the AM scaffold. These findings indicate that the decrease in redox ratio during adipogenic differentiation is associated with fatty acid synthesis and lipid accumulation. Our methods therefore enabled us to identify and measure dynamic correlations between lipid droplet formation and cell metabolic state, while providing insight on the spatial heterogeneity of the observed signals. Such assessments could have a significant impact in the optimization of tissue engineering protocols. In addition, they could serve to assess function of adipose tissues not only in the context of regenerative medicine but also for disease diagnostics and drug screening efficacy.

8948-68, Session 11

Video-rate cellular imaging of whole embryo with extended-field two-photon light-sheet microscopy

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We report a two-photon imaging technique that performs cellular-resolution video-rate imaging in deep tissue over an extended field of view of 500-by-500 microns. The technique utilizes scanning Bessel

beam light sheet illumination to generate large-area two-photon images at video rate. Deep tissue imaging was performed on live zebrafish embryos, whose entire GFP-labeled vasculature in the head was captured at 2-micron isotropic resolution, and real-time heartbeat and blood circulation were captured at video rate. The instrument is capable of continuous lateral zooming to a maximal resolution of 0.5 micron, which is suitable for fine structure imaging such as hindbrain neurons of zebrafish embryos and axons of drosophila embryos. The method generates minimal photobleaching and toxicity, and is capable of perform long-term time-lapse 3D imaging of developing embryos.

8948-69, Session 11

Optimized delivery of femtosecond laser pulses through the hollow-core photonic crystal fiber for the temporally focused wide-field two-photon endomicroscope

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Hollow core photonic crystal fiber (HCPCF) is advantageous for delivering femtosecond pulses for the temporally focused wide-field two photon endomicroscope (TFWTPE) since the air core minimize such effects as self-phase modulation and group velocity dispersion (GVD) and helps to maintain the temporal shape of the input pulses. Although, two photon excitation can be greatly enhanced with high peak power and low repetition rate pulses from the regenerative amplifier, this poses a great challenge since the fiber can be easily damaged from the thermal or ionization effect caused by the extremely high intensity focused beam at the inlet of the fiber. In this paper, we propose a strategy of delivering optimized femtosecond laser pulses through HCPCF for imaging biological sample with TFWTPE using the pulse splitter for multiplying the repetition rate and the pulse compressor for compensating GVD. A previous study showed that increasing the repetition rate while maintaining the pulse intensity can increase the signal rate of the biological sample with small two photon excitation cross section. In addition, increasing the repetition rate while decreasing the pulse intensity proportionally can reduce the photodamage. In the context of the delivery of the amplified pulses through the fiber, increasing the repetition rate can deliver higher rate of pulses while reducing the chance of damaging the fiber. GVD which mostly caused by the waveguide dispersion in HCPCF is compensated by the single prism type pulse compressor. This has additional benefit of further reducing the peak power at the inlet of the fiber.

Conference 8949: Three-Dimensional and Multidimensional Microscopy: Image Acquisition and Processing XXI

Monday - Thursday 3 –6 February 2014

Part of Proceedings of SPIE Vol. 8949 Three-Dimensional and Multidimensional Microscopy: Image Acquisition and Processing XXI

8949-55, Session PMon

Simplified methods of design, implementation, and characterization of a spectrometer-based FD-OCT

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In this work, we report simple optical design of a high speed and high spectral resolution spectrometer based on the first order calculation. The spectrometer was design and optimized for high speed detection of spectral interference signal for 3D FD-OCT applications. We then detailed the hardware implementation of the system in our laboratory at Suranaree University of Technology, Thailand, by utilizing only off-the-shelf optical components. The current spectrometer setup was used for high speed capturing of the spectral interference fringes at up to the camera speed limit of 70,000 spectra per second, enabling cross-sectional microscopic imaging of biological samples of more than 100 frames per second (for a 500 depth scans per frame). In addition, we reported several simple yet robust techniques for characterization of the system performance (e.g. axial resolution, lateral resolution, system sensitivity, depth of imaging, and spectral resolution) in the context of FD-OCT 3D imaging. The development of this high speed and high resolution NIR spectrometer is part of our ultimate goal to develop a prototype of a research-grade FD-OCT system that provides better imaging speed and resolution in comparing to available commercial OCT systems at relatively lower cost. The design of low-cost, high performance FD-OCT system would make the technology widely accessible to other researchers in the field of biomedical research and related areas in Thailand in the next few years. We strongly believe that this research and development would lead to self-reliance and sustainable technology development in the country.

8949-56, Session PMon

Automatic segmentation of fluorescence lifetime microscopy images of cells using multi-resolution community detection

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Inspired by a multi-resolution community detection (MCD) based network segmentation method, we have developed an automatic method for segmenting fluorescence lifetime (FLT) imaging microscopy (FLIM) images of cells. The image processing problem is framed as identifying segments with respective average FLTs against the background in FLIM images. The proposed method segments a FLIM image for a given resolution of the network defined using image pixels as the nodes and similarity between the FLTs of the pixels as the edges. In the resulting segmentation, low network resolution leads to larger segments, and high network resolution leads to smaller segments. Further, using the proposed method, the mean-square error (MSE) in estimating the FLT segments in a FLIM image was found to consistently decrease with increasing resolution of the corresponding network. The MCD method outperformed a popular spectral clustering based method in performing FLIM image segmentation. At high resolution, the spectral segmentation method introduced noisy segments in its output, and it was unable to achieve a consistent decrease in MSE with increasing resolution.

8949-57, Session PMon

Super-resolution differential interference contrast microscopy by structured illumination

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Differential interference contrast (DIC) microscopy has been one of the most widely used techniques and gained broad application in the biomedical community. Compared to Zernike phase contrast (PhC) microscopy, DIC has several benefits, including high spatial resolution owing to the full numerical aperture utilization, free of halo and shade-off artifacts and optical sectioning capability. However, since DIC microscopy is one kind of lens-based far-field light microscopy, its spatial resolution is limited by the law of diffraction. This makes many biological structures smaller than the diffraction limit unresolvable. Therefore, the enhancement of spatial resolution below the classical diffraction limit is critically important to reveal the finer structures for an increasing understanding of their functions. Inspired by the recent progress in structured illumination super-resolution microscopy , we present a structured illumination differential interference contrast (SI-DIC) microscopy to break the diffraction resolution limit of the DIC microscopy. The idea is to apply the concept of structured illumination to the conventional DIC microscopy to expand the bandwidth of coherent transfer function (CTF) of DIC imaging system, and thus reconstruct DIC image with resolution significantly better than the diffraction limit. With 0.8 numerical aperture condenser and objective, the reconstructed SI-DIC image of 53 nm polystyrene beads reveals lateral resolution of approximately 190 nm, doubling that of the conventional DIC image. We also demonstrate biological observations of label-free cells with improved spatial resolution. The SI-DIC microscopy can provide sub-diffraction resolution and high contrast images with marker-free specimens, and has the potential for achieving sub-diffraction resolution quantitative phase imaging.

8949-58, Session PMon

Two-frequency laser scanning confocal fluorescence microscope for specimen-induced spherical aberration cancellation

Yung-Chin Chung, Jheng-Syong Wu, Chien Chou, Chang Gung Univ. (Taiwan)

Our previous research proposed a two-frequency Zeeman laser scanning confocal microscope (ZLSCM) and experimentally demonstrated its ability of reducing the spherical aberration produced by the refractive-index mismatch in the medium based on coherence heterodyne detection. These are due to the features of ZLSCM by (1) a common-path propagation of two-frequency linearly polarized waves, (2) the spatial coherence gating, polarization gating and spatial filtering gating and (3) the heterodyne detection. In this study, the fluorescence ZLSCM (F-ZLSCM) was setup where its fluorescence axial response was measured via intensity modulation of fluorescence signal detection. The performance of F-ZLSCM compared with fluorescence conventional laser scanning confocal microscope (F-CLSM) was demonstrated. Finally, when probing a thick specimen, an improvement on the section image by F-ZLSCM is anticipated and discussed.

8949-59, Session PMon

New light field camera based on physical based rendering tracing

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Even though light field technology was first invented in the 20th century, it did not gain popularity due to the limitation imposed by the computation technology. With the rapid development of computer technology over the last decade, the limitation has been uplifted and the light field technology quickly returns to the spotlight of the research stage. In this paper, PBRT (Physical Based Rendering Tracing) was introduced to overcome the limitation of using old optical simulation approach to study the light field camera technology. More specifically, traditional optical simulation approach can only present light energy distribution but typically lack the capability to present the pictures in realistic scenes. By using PBRT, which was developed to create virtual scenes, 4D light field information was obtained to conduct initial data analysis and calculation. This PBRT approach was also used to explore the light field data calculation potential in creating realistic photos. Furthermore, we integrated the optical experimental measurement results with PBRT in order to place the real measurement results into the virtually created scenes. In other words, our approach provided us with a way to establish a link of virtual scene with the real measurement results. Several images developed based on the above-mentioned approaches were analyzed and discussed to verify the pros and cons of the newly developed PBRT based light field camera technology. It will be shown that this newly developed light field camera approach can circumvent the loss of spatial resolution associated with adopting a micro-lens array in front of the image sensors. Detailed operational constraint, performance metrics, computation resources needed, etc. associated with this newly developed light field camera technique are presented in detail.

8949-60, Session PMon

Real-time stroboscopic full-field optical coherence tomography based on graphics processing unit

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We present the real-time stroboscopic full-field optical coherence tomography (FF-OCT) system that is based on graphics processing units (GPU). The basic configuration of the proposed FF-OCT system was the Linnik interferometer. While scanning of a reference mirror in the axial direction, a series of the transverse sectional image was captured with a 2-dimensional CCD camera. To get the depth-resolved 2-D image, the light source of OCT was turned on and off like a stroboscope at the Doppler frequency, generated by scanning the reference mirror, of the OCT system. The CCD camera used in experiment operated at a rate of 200 frames per second, but the Doppler frequency was ~kHz. To overcome the slow operation of the CCD over the Doppler frequency, the light source was operated in the stroboscopic mode. In addition, lock-in detection technique was utilized in order to avoid the dissolution of the coherent signals during the integration time of the CCD. Furthermore, the Doppler frequency shift due to nonlinear scanning motion of the reference mirror was monitored by using an auxiliary interferometer and then fed back to the light source driver so that the strobe frequency was always matched with the Doppler frequency of the OCT system. For the real-time 3-D rendering, we used a graphics processing units to reconstruct data sets acquired at a high speed.

8949-61, Session PMon

A new concept of stereoscopic imaging system using single optical channel and a deflector: pilot study

Won Hyuk Jang, Heesung Kang, Taeyoon Son, Jihoon Park, Eunkwon Jun, Byungjo Jung, Yonsei Univ. (Korea, Republic of)

In present study, a possible development of new concept of stereoscopic imaging system using single optical channel with single detector was presented. Use of single detector and a transparent rotating disk (TRD) could overcome the limitations raised from conventional three-dimensional (3D) system. TRD generates left and right image disparity, in relation to TRD rotation angle. The feasibility of rotation angle was analyzed both theoretically and experimentally. To achieve a real-time detection, an automated rotation method was applied and the visualization of 3D image to end-user was achieve using active shutter method. Developed system could replace conventional 3D imaging system and its possible applications include medicine, entertainment and other imaging application.

8949-62, Session PMon

High-resolution volumetric cell imaging based on interferometric multiple wavelength phase imaging technique

Jae Seok Park, Korea Photonics Technology Institute (Korea, Republic of); In Hee Shin, Hyeong Ju Park, Joo Beom Eom, Byeong-il Lee, Korea Photonics Technology Institue (Korea, Republic of)

Phase imaging techniques have great potential for developing specialized microscopes, as they permit several abilities to be performed at unprecedented resolution. Those techniques may not only replace current optical microscopes but offer a number of significant advantages such as functional and volumetric digital imaging. In biological applications, 2pi ambiguities have been main limitation but recently software or hardware based methods are introduced to overcome the constraint.

In this research, we report high resolution volumetric cell imaging based on interferometric multiple wavelength phase imaging technique using our low coherence phase microscopy system. A home-made multi-band broadband source provides separate spectral data, which can be easily applied for multiple wavelength phase imaging and then result in more increased measurement range. Our low coherence phase microscopy reduced environmental vibration and system noises. The axial resolution is still lower than 10 nm while axial measurement range is higher than 10 ?m. The high ratio permits to find malignancy in surrounding normal cells. It can also help discriminate normal appearing cells from the suspicious cancer cite. The results may be great potential for imaging cellular structures, especially for visualizing abnormal cancer cells with volumetric rendering.

8949-63, Session PMon

Experimental analysis of focal fields in laser scanning fluorescence stereomicroscopy

Yan Long Yang, Xi'an Institute of Optics and Precision Mechanics (China); Tong Peng, Xi'an Institute of Optics and Precision Mechanics (China); Ming Lei, Xing Zhou, Runze Li, Di Wu, Baoli Yao, Xi'an Institute of Optics and Precision Mechanics (China); Tong Ye, The Univ. of Alabama at Birmingham (United States)

Stereoscopic techniques are generally used as post processing

methods to flatten three dimensional data into two dimensional views for visualization with depth perception. For example, in confocal microscopy, an acquired collection of image slices can be presented as a series of stereoscopic pairs that are easily interpreted as a 3D image by human vision. The direct method to generate the stereoscopic images involves laterally shifting either samples or imaging lens. We reported earlier that tilted Bessel beams could generate stereoscopic images in laser scanning two-photon fluorescence microscopy. However, the tilted Bessel beams may experience serious aberrations and become less useful for imaging. In order to fully understand and to optimize the imaging system, we perform theoretical and experimental analysis on the light focusing process of Bessel beams with real-world optical elements. The stereoscopic image formation is simulated numerically and demonstrated experimentally.

8949-64, Session PMon

Selective plane illumination microscopy with structured illumination based on spatial light modulators

Runze Li, Xing Zhou, Di Wu, Tong Peng, Yan Long Yang, Ming Lei, Xi'an Institute of Optics and Precision Mechanics (China); Xianhua Yu, Xi'an Institute of Optics and precision Mechanics?Chinese Academy of Sciences (China); Baoli Yao, Xi'an Institute of Optics and Precision Mechanics (China); Tong Ye, The Univ. of Alabama at Birmingham (United States)

Structured-illumination microscopy (SIM) is an efficacious tool to decrease the contribution of the out-of-focus light to images of specimens. However, in SIM, the frequency of the spatial modulation applied to specimens should be adjustable according to the optical properties of the specimens to reach the optimal contrasts. Hence, a common theme in SIM is how the flexibility and quality of modulations at different frequencies can be improved. Digital scanned laser light-sheet microscopy with structured illumination (DSLM-SI) has been the most flexible means for generating modulation and optical sectioning. The complexity of synchronization between the temporal modulation and the beam scanning makes it hard to use and less stable; it also spends more time to acquire a single plane information than selective plane illumination microscopy(SPIM). In this report, we present a recent effort to use a spatial light modulator (SLM) to provide spatial modulation in SPIM. With the SLM, both of the frequency and phase of lateral modulation can be changed rapidly; moreover, this SLM-based SPIM can achieve fast imaging without mechanical moving parts. We demonstrate that our setup allows faster image acquisition comparing with DSLM-SI.

8949-65, Session PMon

Beam propagation in superresolution microscopy: a three-dimensional simulation in biological cells

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The recent development of far-field super-resolution fluorescence based imaging methods has proven the ability to improve the spatial resolution to sub-100 nanometers. Among many super-resolution methods, stimulated emission depletion (STED) microscopy has been demonstrated a more practical approach when combination of the imaging speed, penetration depth and ease of sample preparation is considered. Nonetheless, the improvement of spatial resolution in STED microscopy relies on the intensity distributions of the "doughnut"-shaped STED beams, which are often generated by applying phase modulations to the beams. Helical and annular pi-stepped phase masks are two commonly used in STED microscopy and improve lateral and axial resolution respectively. However, both of phase modulations are thought to be susceptible to aberrations and refractive index heterogeneity caused by either imaging systems or specimens. The deteriorated focusing quality may result in reduced spatial resolution or increased laser power exposed on samples, of course, both of which are not expected when imaging biological samples. In this report, we use the finite difference time domain (FDTD) method, a 3D full vector computational method, to simulate the focusing processes of different types of STED beams in tissue models composed of various cellular and subcellular components. The outcomes of the simulations can provide useful information for controlling adaptively aberration and scattering effects and thereby improve the spatial resolution in STED microscopy.

8949-66, Session PMon

Detecting jaundice by using digital image processing

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When strong Jaundice is presented, babies or adults should be subject to clinical exam like "serum bilirubin" which can cause traumas in patients. Often jaundice is presented in liver disease such as hepatitis or liver cancer. In order to avoid additional traumas we propose to detect jaundice (icterus) in newcomers or adults by using a not pain method. By acquiring digital images in color, in palm, soles and forehead, we analyze RGB attributes and chromatic coordinates as the parameter to characterize patients with either jaundice or not, and we correlate that parameters with the level of bilirrubin. By applying support vector machine we distinguish between healthy and sick patients.

8949-67, Session PMon

Using a digital mirror device to enhance the spatial resolution of a microscopy

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As a spatial modulated lighting pattern provider, it is widely believed that a digital mirror device can be a cost-effective alternative for expensive confocal imaging devices. During the past decade, the design of such a microscopic system consisting of a digital mirror device is frequently reported. Though the enhancement of spatial resolution and contrast are expected, convincing images with improved quality are rare to seen. The concrete implementation of a microscopic system consisting of a digital mirror device needs to successfully resolve multiple issues such as the adverse effects caused by the tilt angle of the micro-mirrors contained in a digital mirror device from its base board, the registration between a micro mirror of the digital mirror device and the image pixel of the photo-detector and so on. In this paper, we report the design of a middle body consisting of a digital mirror device which can be an independent attachment to a conventional microscope to convert the latter into a semi-confocal imaging system, in a similar way as a filter-cubic wheel that is placed on a regular microscopy to convert the latter into a fluorescent microscopy. Images of real object with improved spatial resolution and contrast are provided to demonstrate the effectiveness of using digital mirror device as an alternative for constructing a semi-confocal systems. Such a semi-confocal imaging system has many advantages compared to a conventional laser-scanning confocal systems including lower cost and higher imaging speed. In addition, it allows convenient dynamic adjustment between imaging quality and imaging speed.

8949-68, Session PMon

Fast fluorescence holographic microscopy

Wan Qin, Clemson Univ. (United States); Yingying Li, Shenzhen Univ. (China) and Clemson Univ. (United States); Xiaoqi Yang, Clemson Univ. (United States); Xiang Peng, Shenzhen Univ. (China); Xinghua Qu, Tianjin Univ. (China); Bruce Z. Gao, Clemson Univ. (United States)

Holography is an attractive imaging technique as it offers the ability to record a complete three-dimensional (3D) volume in one frame of image. However, holography has not been widely applied to the field of 3D fluorescence microscopy, because fluorescence is incoherent, and creating holograms requires a coherent interferometer system. Although scanning one beam of an interferometer pattern across the rear aperture of an objective to excite fluorescence in a specimen overcomes the coherence limitation, the mechanical scanning is complicated, making the image capturing slow, and the process is limited to use of low-numerical-aperture objectives. In this regard, FINCHSCOPE, a motionless microscopic system based on Fresnel incoherent correlation holography, has been proposed by Rosen and Brooker to record high-resolution 3D fluorescence images of biological specimens. FINCHSCOPE employs three-step phase-shifting interferometry so that it can eliminate the twin image and the bias term resulting from each single hologram. However, since it requires three holograms with different phase-shifting and the phase modulation on the screen of spatial light modulator (SLM) needs to refresh on each acquisition, the entire recording speed is limited. In this study, we developed a unique two-step phase-shifting technique for FINCHSCOPE, in which only two holograms are required for reconstruction of microscopic fluorescence objects. A phase-only SLM was employed to modulate a single fluorescence beam into two beams that can interfere with each other. An EMCCD camera, working in a frame transfer mode, was utilized to record the holograms. The two exposures and the frame transfer that were controlled with two specific sets of parallel register clock sequences were set to match the refreshing rate of the SLM (typically, rise time 5 ms and fall time 10 ms). Therefore, super-fast acquisitions (~50 Hz) of 3D microscopic images were achieved without the need for scanning. This enables real-time observation of three-dimensionally moving fluorescence bio-particles, e.g., migration of mitochondria in cardiomyocytes.

8949-69, Session PMon

Amplitude, phase, and polarization control with a single spatial light modulator

Thomas G. Brown, Michael J Theisen, Stephen Head, Jonathan D Ellis, Univ of Rochester (United States)

Point spread function engineering is usually accomplished by controlling the amplitude, phase and/or polarization of the pupil fields. We analyze and test an optical design for full amplitude, phase, and polarization control of the pupil fields using a single spatial light modulator. In our scheme, the beam is spatially split into four components that represent the in-phase and quadrature components of two orthogonal polarizations. The approach can be used either with state-of-the-art reflective spatial light modulators or using the transmission modulators from retired liquid crystal projectors.

8949-1, Session 1

A high-frame-rate, widefield optical-sectioning microscopy with finer axial resolution than confocal microscopy

Jiun-Yann Yu, Daniel B. Holland, Yun Mou, Marco A. Allodi, Geoffrey A. Blake, Chin-Lin Guo, California Institute of Technology (United States)

We theoretically and experimentally demonstrate a widefield fluorescence imaging technique that achieves finer axial resolution than confocal fluorescence microscopy (CFM) with a simple optical design.

The underlying principle of our technique is the integration of multifocal multiphoton microscopy (MMM) and structured illumination microscopy (SIM). We achieve high-frame-rate operation by using an amplified ultrafast pulse train at a 1 kHz repetition rate as the excitation light, and by adopting multifocal patterns of dense foci spacing so as to illuminate the entire field of view with small amounts of foci translation. Previous studies suggest that the use of a low-repetition-rate pulse train, such as ours, can reduce photo-thermal damages and photobleaching. The closely spaced foci increase unwanted out-of-focus excitation, so we incorporated SIM, utilizing the multifocal pattern to eliminate the out-of-focus signal, into the imaging procedures. Compared with CFM, our technique can achieve ~1.3-fold improvement in axial resolution according to physical optics-based simulations (256nm versus 329nm of CFM with a high-aperture objective); this factor of improvement was also experimentally confirmed. Out-of-focus excitation could be significantly reduced by introducing various time delays to individual foci, as demonstrated by time-multiplexed MMM; we have characterized this effect in a numerical simulation incorporating our higher density of foci.

Besides the superior axial resolution, the operation of our microscope requires only sequential control of individual components, as already built into many microscope automation programs. Time-lapse imaging of living cells demonstrates the high frame rates and low damages of our technique.

8949-2, Session 1

Design and application of the snapshot hyperspectral imaging Fourier transform (SHIFT) spectropolarimeter for fluorescence imaging

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We present a novel and inexpensive Stokes imaging spectropolarimeter based on the Snapshot Hyperspectral Imaging Fourier Transform (SHIFT) spectrometer. A rotating quarter wave plate and stationary linear polarizer placed in front of the SHIFT spectrometer enables us to reconstruct an object's spectra and Stokes parameters in the visible spectrum. Measurements are stored in the form of three-dimensional (3D) Stokes datacubes, which contain the object's spatial, spectral, and polarization information for every pixel location in the 216x216 frame. All spectra and Stokes parameters can be reconstructed in real time given short integration times and rapid reconstruction rates through the graphics processing unit (GPU). We discuss calibration methods, review design considerations, and present preliminary results from proof-of-concept experiments.

8949-3, Session 1

Ultrafast laser scanning confocal microscope with variable aspect ratio field of view

Ki Hyun Kim, Travis Jarrell, Adela Ben-Yakar, The Univ. of Texas at Austin (United States)

We built and tested an ultrafast laser scanning microscopy system with variable aspect ratio field of view (FOV) and pixel number. Depth resolved ultrafast imaging is essential in developing high-throughput

automated examination of three-dimensional, neuronal regeneration in the small animal model, C. elegans after injury. Neither commercial nor academic, custom built laser scanning confocal microscopes provide the required flexibility to image a high-aspect ratio FOV at ultrafast imaging speeds. Our microscopy system accommodates rectangular FOV with any desired aspect ratio and number of pixels that could be acquired at very high speeds limited by the minimum exposure time to collect signal above the noise (~20 ns per pixel). For example, imaging re-growing axons of C. elegans requires a FOV of 350 ?m x 50 ?m with the highest available optical resolution. We therefore use a 1.3 NA, 40 x, and oil immersion objective with corresponding pixel numbers of 3,500 and 500, respectively, to fulfill Nyquist criteria. The FOV in both axes can be freely chosen from 10 ?m to the maximum FOV determined by the objective. The imaging speed of the system is ultimately determined by the resonant mirror as long as enough number of photons can be collected per pixel. For example, the imaging speed for a FOV of 350 ?m x 10 ?m is more than 100 frames per second. The custom software written in LabVIEW controls the whole system and processes data on the fly. We will present images of regenerating axons acquired at high-throughput.

8949-4, Session 1

Modulated alignment dual-axis (mad) confocal microscopy for deep optical sectioning in tissues

Steven Y. Leigh, Ye Chen, Jonathan T. Liu, Stony Brook Univ. (United States)

Our lab is exploring a new strategy to enable optical-sectioning microscopy at unprecedented depths. This technology leverages the inherent strengths of the dual-axis confocal (DAC) microscopy approach for rejecting out-of-focus and multiply scattered background light in tissues, as demonstrated through simulations and experiments. The DAC architecture is unique in that it utilizes an intersecting pair of illumination and collection beams to significantly improve the spatial-filtering and optical-sectioning performance of confocal microscopy. Here, we propose that modulating the spatial alignment of the dual-axis beams at a frequency f, such that signals generated at the focal volume of the microscope are modulated at 2f, can further provide an order-of-magnitude improvement in optical-sectioning contrast. The fundamental reason for this, currently being demonstrated in simulations and experiments, is that out-of-focus and multiply scattered background signal is negligibly modulated in intensity due to the micron-scale spatial modulation of the dual-axis beam alignment, whereas the ballistic signal generated at the focus of the microscope is efficiently modulated. Phase-sensitive lock-in detection may therefore be used to selectively remove background light, thereby enhancing our ability to image deeply within highly scattering tissues. The MAD technique is the first to combine the proven advantages of the DAC architecture with focal-volume modulation to enable unprecedented imaging contrast and depth in tissues.

8949-5, Session 1

Combined spatially chirped modulation and spectral encoding for improved imaging speed in confocal microscopy

Soocheol Kim, Jaehyun Hwang, Jung Heo, Suho Ryu, Chulmin Joo, Yonsei Univ. (Korea, Republic of)

Laser scanning confocal microscopy (LSCM) provides high-resolution and high-contrast images of material and biological specimens in three dimensions. Conventional LSCM operation involves beam scanning in 3D, which inherently compromises image acquisition speed. Spectrally encoded confocal microscopy (SECM) is a type of confocal microscopy that provides faster image acquisition time. SECM employs a diffraction grating to disperse a broadband light and encode one-dimensional spatial information into the wavelengths. Therefore, it does not require beam scanning along the faster axis, only requiring beam scanning in the slower axis. This feature has allowed high-speed and compact LSCM implementation. However, clinical imaging at cellular scales requires even more improvement in speed and implementation to reduce motion artifacts

We developed a new imaging technique (T-SECM) that can potentially improve image acquisition speed of SECM. T-SECM utilizes spectrally resolved line illumination on a specimen. The spatial information in the wavelength-dispersive axis can be measured with spectrally resolved detector, while the coordinates in the other axis are encoded with different modulation frequencies. The reflected light is captured and measured, and spectrally resolved measurement and Fourier decomposition reveal 2D spatial information of the specimen. We implemented a prototype of T-SECM based on a super-continuum light source and a custom built high spectrometer. The spectrometer has a line rate of 70 kHz. The measured field of view was > 500um x 500um, and the lateral resolution was <2 um.

In this talk, we will describe T-SECM concept and implementation, discuss its features, and present T-SECM images of biological tissues.

8949-6, Session 2

Correction of image artifacts caused by refractive index gradients in scanning laser optical tomography

Georgios Christian Antonopoulos, Laser Zentrum Hannover e.V. (Germany); Dimitri Pscheniza, Medizinische Hochschule Hannover (Germany); Raoul-Amadeus Lorbeer, Marko Heidrich, Laser Zentrum Hannover e.V. (Germany); Kristin Schwanke, Robert Zweigerdt, Medizinische Hochschule Hannover (Germany); Tammo Ripken, Heiko Meyer, Laser Zentrum Hannover e.V. (Germany)

We present a technique for correcting image artifacts caused by refractive index distributions in Scanning Laser Optical Tomography (SLOT) and related techniques.

Optical Projection Tomography (OPT) and its offspring SLOT are 3D microscopy techniques for the acquisition of multimodal volumetric images of mesoscopic biological samples with visible light.

Both techniques are fundamentally based on reconstructing the interior of a sample from a set of parallel ray projections acquired over a number of viewing angles. For that reason, refractive index matching of the sample is usually performed to eliminate effects of distortion by refraction and to assure parallel ray projections. Some applications, however, do not allow removing all refractive index changes in the optical path. For instance, in vitro sample containment in a test tube induces an additional layer of glass that cannot necessarily be matched to the refractive index of the culture medium.

We consider the special case of a piecewise constant refractive index distribution with cylindrical symmetry. The gradient is assumed to vanish in the direction of the rotational axis to achieve a set of separable 2D problems. Numerical simulations and experimental results are used to illustrate the connection between the Radon transform and the generalized projection, which accounts for the refractive index distribution. Thereupon we will describe a technique that transforms generalized projections to Radon projections (sinograms) and thus allows artifact free reconstruction within the sample volume.

8949-7, Session 2

Portable advanced digital holographic off-axis camera for quantitative phase microscopy

Zahra Monem Haghdoust, Christophe Moser, Christian D. Depeursinge, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

We propose and experimentally demonstrate a device in which common-path interferometry combined with off-axis holographic geometry is used to realize a digital holographic camera which can be attached to the camera port of a conventional transmission microscope for complex wavefront analysis. A thick transmission volume grating recorded holographically into thick photosensitive glass splits the beam containing the sample information in two beams. The untouched transmitted beam creates the sample arm of the interferometer. The Bragg diffracted order of the grating is spectrally and spatially filtered by diffraction to generate a clean reference beam. Double passing the diffracted order through the grating using a retroreflector device provides filtering in two dimensions. The spatial filtering done by the grating which works based on high angular selectivity of thick volume gratings, reduces the alignment spatial sensitivity which is an advantage over the conventional spatial filtering done by pinholes. Besides, using a second thick grating, we introduce a desired coherence plane tilt in the reference beam which is sufficient to create high-visibility interference over the entire field of view. The full-field off-axis interferograms are created from which the amplitude and phase can be reconstructed. The advantage of the proposed camera is the insensitivity to the alignment, thus can be the basis for a standalone camera mountable on a standard optical microscope.

8949-8, Session 2

Extended penetration depth in optical imaging using array signal processing

Jaeduck Jang, Jaeguyn Lim, Wooyoung Jang, Ji-Yeun Kim, Samsung Advanced Institute of Technology (Korea, Republic of)

Optical coherence tomography (OCT) is a non-invasive optical tomographic imaging technique without exogenous agents. For the last two decades, OCT became standard diagnostic tool in ophthalmological applications. Recently, endoscopic OCT systems are introduced for lung, esophagus, and intra-vascular imaging using miniaturized scanning probr using single mode fiber. Comparing with conventional modalities, OCT has pros and cons which are resolution and penetration depth, respectively. In case of resolution, optical lens and broadband near-infrared light donate up to few micrometer resolutions in axial and lateral directions. While OCT provides micrometer resolutions, its penetration depth is less than few millimeters due to the multiple-light scattering in inhomogeneous tissues. In general, multiple-light scattering gradually decreases signal-to-noise ratio (SNR) in depth, and thus light emitted from OCT does not penetrate in tissue as long as ultrasound.

To improve penetration depth in OCT, we propose array signal processing algorithm to separate signal and noise from multiple measurements. The conventional methods for noise reduction in OCT are spatial/frequency compounding and Fourier analysis. The compounding methods utilize quasi-uncorrelated multiple measurements to equalize noise level, and Fourier analysis reduces noise component in reconstruction step using a kind of Fourier transforms. The proposed method requires multiple measurements with spatial light modulator (SLM) to generate uncorrelated multiple measurements. From multiple measurement vector (MMV) we calculate covariance of MMV for each pixels in single A-scan. Covariance matrix is decomposed into signal and noise subspaces by eigen-decomposition. By using truncated singular value decomposition (tSVD), we finally reconstruct signal-enhanced image without noise subspace.

8949-9, Session 2

An efficient, rigorous model of optical microscopes employing incoherent illumination

Peter R. T. Munro, Lixin Chin, Andrea Curatolo, David D. Sampson, The Univ. of Western Australia (Australia)

Realistic models of image formation are important for designing imaging systems, interpreting images and developing image processing algorithms. The advent of microscopes employing technologies such as high numerical aperture lenses, polarization detection and tomographic reconstruction has required computational models of ever increasing realism to be developed. Such models employ an electromagnetic description of light to model both the optical system and interaction of light with samples. To model general samples, a numerical technique is required to describe the interaction of light with the sample. The finite-difference time-domain (FDTD) method has been employed by multiple groups for this purpose, including in the first rigorous model of coherent optical microscopes.

We will present the details of a newly developed model for simulating image formation in optical imaging systems employing broadband light, which we believe to be the first of its kind. System parameters such as numerical aperture, source polarization and source bandwidth can all be simulated for general samples which may be dispersive. The model has, at its core, the FDTD method and its strength lies in several key approximations that enable image formation to be modeled without the need for high-performance computing. We will describe, in detail, the approximations required to efficiently model image formation using broadband light and the effects of such approximations. Examples of simulated optical coherence tomography images will be given, along with comparisons with experimentally acquired images. We will also demonstrate some potential artifacts which may result when using the FDTD in such a manner.

8949-10, Session 3

Sequential erosion tissue imaging (SETI)

Benjamin L. Cox, Morgridge Institute for Research (United States) and Lab. for Optical and Computational Instrumentation (United States); Jeremy S. Bredfeldt, Lab.for Optical and Computational Instrumentation (United States) and Morgridge Institute for Research (United States); Kevin W. Eliceiri, Thomas R. Mackie, Lab. for Optical and Computational Instrumentation (United States) and Morgridge Institute for Research (United States)

Many biological problems necessitate high resolution, accurate three-dimensional (3D) visualization. Traditional 3D biomedical imaging is largely limited to large spatial scale with whole body medical imaging, such as CT, and MRI, or to micro scale optical sectioning methods such as confocal and multiphoton laser scanning microscopy techniques. There is great need for 3D imaging of large volumes at micron level resolution. We have developed a technique to address this need, termed Sequential Erosive Tissue Imaging (SETI). SETI can capture large fields of view of 1-2cm3 at a resolution of 1.5 microns in the x-y plane and 2.5 microns in the z-dimension.

During the SETI process, thin layers of tissue within a sample are precisely removed, and each newly revealed surface is imaged. We have developed a prototype fluorescence system that employs the SETI process. This prototype uses a miniature 3 axis mill, LED illumination, low light imaging CCD camera, modular infinity optics, and 4X .4 NA objective . This imaging system currently produces pixel sizes of 1.5 microns and a field of view of 1.9 mm by 1.5 mm. Field of view can be increased by the capture of multiple images at each surface, then stitching the images together.

This system may be generally applied to 3D microscopic imaging,

with the caveat that samples will be destroyed in the process. We present high resolution 3D images of several tumor samples showing both fluorescently labeled and intrinsic contrast. Image acquisition automation was performed with AutoIT freeware (autoitscript.com). Image processing, including stacking, stitching, and contrast enhancement was performed with FIJI (http://fiji.sc).

8949-11, Session 3

Numerical spherical aberration correction method using spatial light modulator under deep-part fluorescence observation

Yu Takiguchi, Hisayoshi Takamoto, Hamamatsu Photonics K.K. (Japan); Masamitsu Kanada, Hamamatsu Univ. School of Medicine (Japan); Takashi Inoue, Naoya Matsumoto, Hamamatsu Photonics K.K. (Japan); Susumu Terakawa, Hamamatsu Univ. School of Medicine (Japan)

We have developed a confocal fluorescence laser scanning microscopy (CFLSM) incorporating a liquid crystal on silicon spatial light modulator (LCOS-SLM). To achieve high-resolution and high-contrast imaging for deeper part of the tissue with CFLSM, high numerical aperture objective lenses are required to tightly focus excitation light to meet Rayleigh limit(criterion) for the specimens. However, mismatch of refractive index at the boundary of interfacing materials, such as atmosphere, glass cover, and biological tissues, causes spherical aberration. Recently, we proposed a numerical method for correcting spherical aberration. In this method a pre-distorted wavefront pattern for aberration correction is calculated by ray tracing from a hypothetical focal point inside a specimen to the pupil plane. The resulting microscope can correct such spherical aberration.

We observed 6.0?m fluorescent micro-beads dispersed three-dimensionally in agarose gel to confirm effectiveness of aberration correction. We reconstructed a three-dimensional image by taking 20 images with 1 ?m interval near the depth and stacking them. It was apparent that the longitudinal/depth resolution was improved and that the intensity of fluorescence image was increased with aberration correction.

While this method is applicable to other laser scanning microscopes, it has potential to enhance the signals for various super-resolution microscopic techniques, such as stimulated- emission-depletion (STED) fluorescence microscopy.

8949-12, Session 3

The Gray Institute open microscopes applied to radiobiology and protein interaction studies

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We describe an 'open' design methodology for wide-field fluorescence, confocal and fluorescence lifetime microscopy (FLIM) [1], and how the resulting microscopes are being applied to radiation biology and protein activity studies in cells and human tissue biopsies. The design approach allows easy expansion as it moves away from the use of a monolithic microscope body to small, commercial off-the-shelf and custom made modular components. Details have been made available under an open license for non-commercial use at http://users.ox.ac.uk/~atdgroup.

Two radiobiology 'end-stations' have been constructed which enable fast radiation targeting and imaging of biological material opening up completely novel studies, where the consequences of ionising radiation (signaling and protein recruitment) can be studied in situ, at short times following irradiation. One is located at Surrey University, UK [2], where radiation is a highly focused in beam (e.g. protons, helium or higher mass ions). The second is installed at the Gray Institute linear accelerator facility, Oxford University, which uses sub-microsecond pulses of 6 MeV electrons.

FLIM capabilities have enhanced the study of protein-protein interactions in cells and tissues via Förster Resonance Energy Transfer (FRET). Extracting FRET signals from breast cancer tissue is challenging because of endogenous and fixation fluorescence [3,4]; we are investigating novel techniques to measure this robustly. Information on specific protein interactions from large numbers of patient tumors will reveal prognostic and diagnostic information.

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8949-13, Session 3

Characterizing and optimizing the tissue-imaging performance of confocal microscope architectures via monte-carlo scattering simulations

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Confocal microscopy is one of the most pervasive optical imaging techniques, providing high-resolution optical sectioning of cells and tissues. Various confocal microscope architectures have been developed for in vivo tissue imaging, including single-axis confocal (SAC) and dual-axis confocal (DAC) configurations utilizing both point-scanning (PS) and line-scanning (LS) approaches. While it is known that these design variations lead to tradeoffs in imaging performance, a quantitative comparison of the imaging performance of these configurations in highly turbid media would be of value. Here, we perform Monte-Carlo simulations to evaluate the optical-sectioning capability of these various confocal microscope architectures. Our study indicates that, in either PS or LS mode, DAC microscopy exhibits superior rejection of out-of-focus and multiply scattered background light compared to conventional single-axis confocal (SAC) microscopy. In terms of optimizing the design of a DAC microscope, it is generally recognized that the resolution and contrast of a DAC microscope depends on both the crossing angle of the DAC beams, 2?, and the focusing numerical aperture (NA) of the individual beams, ?. However, a detailed study to investigate these dependencies has not been performed. Thus, we utilize Monte-Carlo scattering simulations and diffraction theory calculations to assess the performance of a DAC-PS microscope and a DAC-LS microscope as a function of ? and ?. Results of our study can be used for guiding the optimal designs of DAC-PS and DAC-LS microscopes.

8949-14, Session 3

Adaptive optics in microscopy

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Wide field and confocal fluorescence microscopy are the major tools in biological studies. The oil immersion objectives with high magnification and numerical aperture are widely used to image cells and tissues. Unfortunately, the fluorescence signal diminishes rapidly when deeper

layers of the sample are imaged. The light is being scattered and re-absorbed, and altered by aberrations. The main type of aberrations appearing with oil immersion objectives imaging water based samples is spherical aberration caused by the refractive index mismatch between oil and water. Already at a depth of 5µm the amount of spherical aberration reaches the amplitude of 60nm RMS and reduces dramatically the fluorescence signal. Adaptive optics, using photo-sensors and phase modulator are able to determine the amount of aberrations, correct for them and almost restore the fluorescence intensity. We determined the depth dependence of the amount of spherical aberration by two different methods. The guide star method uses a fluorescent bead embedded at different depth in agar and the wavefront sensor measures directly the aberrations. For the same task we also employed iterative algorithms, like the 3N algorithm [1]. Here will discuss all the usability and limitation of those adaptive optics techniques in microscopy. As a conclusion we will propose the strategy to introduce effectively adaptive optics in microscopy by combining those techniques.

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8949-15, Session 4

Computational lensfree color microscopy for wide field-of-view imaging

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For centuries, lenses have been an inherent part of microscopy; through lenses, the microscope is able to magnify and image objects with high-resolution. However, lenses also pose fundamental limitations to the microscope's performance, mainly a limited depth-of-field and a narrow field-of-view. Lensfree holographic imaging is a computational microscopy method that addresses these limitations by removing the lenses from the optical set-up and replacing them with phase-recovery and wave propagation algorithms to undo diffraction. In its state-of-the-art implementation, lensfree on-chip imaging provides a large field-of-view (e.g., 20-30mm^2) and a high spatial resolution, reaching a numerical aperture of 0.9 across the entire field-of-view. One of the remaining challenges for lensfree holographic microscopy has been to obtain accurate color imaging of specimens, since holographically reconstructed color images are typically distorted, exhibiting a rainbow-like spatial artifact. Color imaging is critical for various biomedical imaging applications since color stains provide contrast and enable the separation of different cell types within a given specimen. Here we introduce and compare two computational methods to mitigate this rainbow-like color noise in lensfree holographic microscopy and achieve an accurate color image without degrading the resolution of the image. Our first colorization method is based on averaging the color information of the image in the YUV color space and the second method is based on Dijkstra's shortest path algorithm. We demonstrate the success of these colorization techniques by imaging stained Papanicolaou smears over a wide field-of-view of 20mm^2 with sub-micron spatial resolution and accurate color reproduction.

8949-17, Session 4

High-contrast 3D microscopic imaging of deep layers in a biological medium

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We have developed a label-free 3D microscopic system to enhance the lateral and axial resolution in imaging of biological organisms. Implementing a dark-field imaging approach, a novel scheme of an opposed-view digital holographic microscope (DHM) has been introduced. DHM has the unique ability to digitally record the 3D information of the amplitude and phase distribution of the optical wave-field within the whole object. Numerical processes allow volumetric reconstruction of the wave-field with sub-micron resolution. The image contrast has been significantly improved for transparent specimens, using the dark-field mode. The coherent noise, introduced by the scattered light, has been suppressed utilizing speckle-field illumination and by averaging over various number of speckle-fields. The optimum number of required speckle-fields and the signal to noise ratio have been also discussed. The system includes a bright-field DHM, extracting the quantitative phase information that can be used to digitally correct the optical aberrations in the system and to reveal the optical thickness of the specimen. Creating aberration and blocking some spatial frequencies, the upper layers of the specimen reduces the image quality for deeper layers. Using the opposed view system, the structures from these layers are better resolvable, which enhances the resolution of the 3D imaging. The method is non-invasive, as the specimen will not be stained and there is no need to fix the sample on a substrate.

8949-18, Session 4

Self-interference fluorescence holography system with high signal-to-noise-ratio and violation of the lagrange invariant

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Fluorescence is widely used in biology because of its ability to make use of specific labeling for both structural and functional imaging. Compared with conventional fluorescence detection methods, holography is attractive because of its ability to detect full three-dimensional information without scanning. However, it is hard to achieve fluorescence holography, since fluorescence is marginally coherent, unlike the laser light. In recent years, a powerful method called Fresnel incoherent correlation holography (FINCH) has been proposed to realize holographic fluorescence detection. However, in conventional configuration, the detector has to be placed far away from the imaging focal plane. As we know, the intensity is strongest in the focal plane, and becomes weaker when away from it. The fluorescence itself is a weak signal, and large detection distance will markedly reduce the signal-to noise ratio (SNR) of the image. Therefore, fluorescence detection in or near the focal plane is necessary.

Here we present a system that can realize near focal plane detection with high extent of interference benefit from its small optical path difference (OPD). This is achieved by set the focal length of the lens for reference wave close to that for object wave. The signal-to-noise ratio (SNR) is improved up to ~21 times comparing with the conventional technique, and weaker signals can be detected. Moreover, we found this system has a more important character of self-interference. In this kind of self-interference holography (SH) system, the Lagrange invariant is violated. This violation might lead to the improved sub-diffraction resolution.

8949-19, Session 4

Study of self-interference incoherent digital holography for the application of retinal imaging

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Self-interference incoherent digital holography (SIDH) is recently developed incoherent digital holographic imaging technique which uses the interference between two copies of waves emanating from each point source of the object. Because SIDH uses the incoherent light as an illumination source, the recorded image is free from the speckle noise which usually appears in the coherent holographic imaging. With the ability to compensate the aberration using the guide star hologram, the investigation of the retina structure can be considered as one important

application of SIDH.

For this purpose, the optical setup of SIDH is configured similar to the Michelson interferometer with two mirrors having different curvatures. However, in the recorded image, the nature of incoherency washes out the interference pattern comes from each point spread function (PSF). Using the phase-shift-interferometry, the complex hologram can be retrieved from the recorded intensity images.

Though the refocused images can be obtained by simply applying the computational propagation such as angular spectrum method, the correlation with the guide star hologram can retrieve the object image from the complex hologram even for the case where the aberration exists. Surprisingly, the correlation method is known to acquire better image quality in terms of SNR and the resolution. We already investigated such features from the series of preliminary experiments conducted to the sample of bovine eye.

The usefulness of applying SIDH for the retinal imaging will be studied with the cone mosaic images obtained from bovine model eye experiment.

8949-20, Session 4

Flash extreme ultraviolet holographic microscopy in a table top setup

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We describe the implementation of single shot extreme ultraviolet (EUV) Fourier transform holographic microscopy setup. The system is capable of 1.2 ns temporal and ~100nm spatial resolutions. A zone plate optic was used as a beam-splitter. The incident beam impinged on the zone plate and then split into multiple orders. The first order focus was used for the reference wave and a central opening in the zone plate allowed the incident beam to directly illuminate the object. The object and reference waves interfered and was collected on an X-ray CCD. The laser source in this holographic system is a compact EUV laser emitting a highly coherent beam at ? = 46.9 nm. The hologram was recorded by a Peltier cooled CCD camera with a 26.7?26.7 mm2 CCD chip, having 13.5 ?m wide square pixels and a total array size of 2048?2048 pixels. The set up was tested utilizing as object an array of nanopillars.

Flash holographic images allowed for the assemble of a “movie” of nano-scale pillars oscillating at MHz frequencies. The prospects of utilizing this technique to characterize a single molecule detector based on the modification of the oscillatory dynamics of nano-scale pillars will be discussed. Also the progress to implement 3D holo-tomographic images of phase objects will be described.

This project was supported by the Defense Threat Reduction Agency – Joint Science and Technology office for Chemical Biological Defense (Grant No. HDTRA1-10-1-007) and the National Science Foundation Engineering Research Center for Extreme Ultraviolet Science and Technology award EEC 0310717.

8949-21, Session 4

Billion-pixel imaging via Fourier ptychographic microscopy

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We report an imaging modality, termed Fourier ptychographic microscopy (FPM), which computationally extends a system’s resolution beyond the limit defined by its optical elements. By adopting a digital wavefront correction scheme, the FPM method can also digitally extend a microscope’s depth-of-focus and compensate for aberrations associated with its optics. We report the implementation of a FPM prototype with a numerical aperture of 0.5, a field-of-view of ~120 mm^2, and a resolution-invariant depth-of-focus of ~0.3 mm. Billion-pixel color images of histology slides verify its successful operation. The reported PFM procedure may transforms the general challenge of high-throughput, high-resolution microscopy from one that is coupled to the physical limitations of the system’s optics to one that is solvable through computation.

8949-22, Session 5

Quantifying melanin distribution using pump-probe microscopy and a 2D morphological autocorrelation transformation for melanoma diagnosis

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The incidence of cutaneous melanoma is rising faster than any other type of cancer, however many experts believe this is due to an increase in false positives rather than an increase in the true occurrence of melanoma. This has motivated the development of pump-probe microscopy, a quantitative molecular imaging technique that provides contrast between diagnostically relevant pigments, namely eu- and pheo-melanin. This nonlinear optical imaging technique yields chemical information by probing the excited state dynamic properties of pigmented molecules and achieves spatial resolution at the sub-cellular level.

Recently, we have demonstrated the ability to quantitatively differentiate between melanocytic nevi and malignant melanomas based on bulk percent eumelanin, without considering its spatial distribution. However, pump-probe microscopy offers a wealth of additional information based on the microscopic distribution of the melanin content. In this work, we present a method based on mathematical morphology to quantify eu-, pheo- and total melanin image structure for melanoma diagnosis. Note that this chemical information is not available with other methods. The structural analysis applies a 2D autocorrelation function and utilizes statistical parameters of the corresponding autocorrelation images—specifically, second moments and entropy—to parameterize structure. Along with bulk melanin chemical information, results show that this method can differentiate invasive melanomas from non-invasive and benign lesions with high sensitivity and specificity (92.3% and 97.5%, respectively, with N = 53 unstained, thin cutaneous lesions). The mathematical method and the statistical analysis will be described in detail and results from cutaneous and ocular conjuctival melanocytic lesions will be presented.

8949-23, Session 5

Compressive sensing spectral domain optical coherence tomography with dispersion compensation

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Applications of compressive sensing (CS) on optical coherence tomography (OCT) have been studied by many groups. In this paper, we propose a novel method which incorporates dispersion compensation to the CS reconstruction of spectral domain OCT (SDOCT) signal. We first show that dispersion compensation can be implemented by multiplying the frequency-dependent correcting phase to the real whole spectra, eliminating the need for constructing complex component of the real spectra. Then A-scan with dispersion compensation can be obtained by multiplying the dispersion correcting phase to the under-sampled spectral data before the CS reconstruction. The under-sampled linear-in-wavenumber spectral data is obtained by sampling from the

original k-space data of a 2048-pixel SDOCT with the pre-generated k-linear sampling mask. We also implement fast CS reconstruction by taking the advantage of fast Fourier transform (FFT). The matrix-vector multiplication that is used a lot in the CS reconstruction is implemented by first zero-filling the vector to full length, then applying FFT to the full length vector. Comparing to the CS reconstruction with matrix multiplication, our method can obtain A-scans with dispersion compensation with a speedup of 48 times, regardless of the sampling rate. Experimental results on both simulation data and real SDOCT data show that proposed method can achieve high quality image with dispersion compensation.

8949-24, Session 5

Analysis of phase conjugation in a turbid medium

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The ability to focus light in most tissue degrades quickly with depth due to high optical scattering. Recently, researchers have found they can concentrate light tightly despite these scattering effects by using a “guidestar” and optical phase conjugation to focus light to greater distances in tissue. An optical or “probe” signal is transmitted through a scattering medium and its resulting wavefront is detected. The wavefront is then conjugated and utilized as a new optical source or “delivery” wave that focuses back to the guidestar’s location with minimal scattering. The power in the delivery wave may be greatly increased for enhanced energy delivery at the focus. Modulation by an ultrasound (US) beam may be utilized to generate the guidestar dynamically and allow for US—resolution at depths of several millimeters.

The delivery wave is successful at focusing light back at the guidestar because it creates constructive interference at the desired focus. However, if the phases of the field contributions change, we expect the delivered power at the focus to be reduced. This paper will analyze the robustness of this method when the probe beam is at one wavelength and the delivery wave is at another. This will allow us to characterize the deleterious effects of varying the phase contributions at the focus.

8949-25, Session 5

Image reconstruction methodologies for structured light based laser sheet microscope for thick tissue imaging

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Three-dimensional (3D) thick tissue fluorescence imaging has numerous applications in biological sciences. Background haze is common in conventional wide-field based fluorescence microscopy systems due to their no depth discrimination capability. Laser sheet based microscopy, where camera is oriented perpendicular to the light sheet, involves effective active illumination at selected axial plane and thus it enables optical sectioning, additionally sample is exposed to less optical radiation. Nevertheless, background haze still remains in laser sheet based microscope, provides limited visibility of sample features. We implemented structured illumination-based methods in laser sheet microscope to reject the background haze, visualizing more clearly the focussed fluorescent-plane. In this paper, we present the image reconstruction methods for structured illumination-based laser sheet microscope applied to thick 3D tissue imaging. Limitations of conventional structured illumination based demodulation methods, called 3Phase and HiLo, are analyzed and a new demodulation method, called 3D HiLo, is presented. Further, maximum likelihood estimation based photon reassignment method is presented for 3D visualization of biological tissues, which provides higher signal-to-noise (SNR) and signal-to-background (SBR) ratios of the reconstructed image. This method seeks to better utilize the volumetric photons by using the ‘prior knowledge’ about the optical transfer function of the system, and provides the reassignment of fluorescence photons generated from off-focal plane excitation.

8949-26, Session 5

Further developments in addressing depth-variant 3D fluorescence microscopy imaging

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3-D imaging with optical sectioning widefield microscopy [1] uses computational methods to provide a better approximation of the fluorescence distribution by addressing the effect of defocus and sample-induced aberrations. Inverse algorithms [2] [3] [4] improve resolution, sharpness and clarity. Practical inverse algorithms need to be accurate as well as fast. Model-based algorithms rely on an accurate representation of the image formation model.

Forward imaging models based on a depth-varying point-spread function (DV-PSF) leads to a substantial improvement in the resulting images because they account for depth-induced aberrations present in the imaging system [5]. PSFs at every layer can be represented using their principle components. Principle component analysis (PCA) representation of the axially varying point spread functions (PSFs) requires fewer convolutions to generate the forward image than the strata based algorithms [6]. It has been shown that an accelerated maximum likelihood image restoration algorithm with a conjugate gradient iteration scheme (specific for Gaussian or Poisson noise models) with a modified Tikhonov method for regularization gives increase in processing speed and faster convergence over the Expectation Maximization algorithm [7].

In our paper we combine the accuracy as well as the faster processing from the PCA representation of DV-PSFs with the fast convergence of the conjugate gradient algorithm to create a PCA-based DV-CG algorithm. Results from both simulated and experimental data from a fluorescence microscope are presented and demonstrate the fast convergence and accuracy of this algorithm.

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8949-27, Session 6

Defocus-based quantitative phase imaging by coded illumination

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Recovering amplitude and phase from intensity images non-interferometrically entails capturing at the least two intensity images under different conditions (e.g. defocus[1,2,3], wavelength [4], or source position [5]). Propagation based phase imaging methods are the most popular, taking multiple images at different defocus planes and reconstructing phase from knowledge of the propagation transfer function via either the Transport of Intensity equation [3] or iterative optimization [2]. This requires either the camera or the object to be moved between measurements, which is difficult to do at high speeds for real-time phase imaging and tomography. Illumination-based phase retrieval methods can avoid moving parts by instead changing the source shape with an optoelectronic device [5]; these however require an aperture in the pupil plane, which is not available in lensless imaging schemes such as X-ray phase tomography systems. We will present a new source-coding scheme for quantitative phase imaging without relying on pupil apertures or moving parts. A theoretical analysis of our method in relation to existing methods is presented within the framework of phase gradient imaging, and a general approach for designing the illumination scheme is provided. The recovered phase from different illumination coding schemes are compared in real experimental conditions with noisy images, and optimal codes are discussed. Our method is capable of numerical reconstruction at real-time frame rates while still being robust and accurate.

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8949-28, Session 6

Near-common-path quantitative phase spectroscopy

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Conventional wide-field QPI permits high-speed phase measurements without the need for lateral scanning. Furthermore, common-path configurations feature high phase stability for low-noise measurements. These capabilities have been exploited in single cell volume measurements in erythrocytes [1], cell growth dynamics [2], and dynamics of pathogen infection [3]. The QPI measurements at multiple wavelengths has been recently applied in enhancing imaging-depth range without 2π phase ambiguity [4] and in quantification of hemoglobin concentrations in erythrocytes [5]. Single-shot quantitative dispersion phase microscope capable of simultaneous wide-field phase measurements at three discrete wavelengths has been reported [6]. Cauchy's equation is typically used to determine dispersion curve from selected spectral points defined by the individual laser sources or by the wavelength selection filters coupled with a broadband source. Recently, quantitative phase spectroscopy based on a rapidly-tunable broadband source has been demonstrated for dispersion measurements with high spectral resolution [7]. In this paper, we report a high-speed quantitative dispersion phase microscope based on near-common-path geometry. The high-speed wavelength tuning will be achieved via a tunable acousto-optic tunable filter coupled to a supercontinuum source. The self-reference approach [6] leads to low-noise phase measurements at many different wavelengths. The feasibility of the approach will be demonstrated in measuring absorption spectrum of the hemoglobin in erythrocytes.

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8949-29, Session 6

Measuring thicknesses of fast dynamic processes using low-coherence interferometric microscopy

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Interferometry can capture the complex wave front containing the amplitude and the quantitative phase of the light interacted with a sample, where the phase is proportional to the optical thickness of the sample. By a smart design of the interferometric system, including the usage of low-coherence illumination sources and common-path geometry of the interferometers, spatial and temporal noise levels of the resulting thickness profile can be sub-nanometric. We present unique low-coherence interferometric imaging techniques for quantitatively tracking the thickness profiles of rapid dynamic processes in up to thousands of full frames per second. The systems are based on compact, portable and easy to align low-coherence interferometers. Using these techniques, we quantitatively imaged rapid dynamics of live biological cells including sperm cells and unicellular microorganisms. We also demonstrate dynamic profiling during lithography processes of microscopic elements, with thicknesses that may vary from several nanometers to hundreds of microns. Finally, we present how rapid interferometry can be helpful for tissue engineering applications, to characterize rapid drug release from skin equivalents in vitro.

8949-30, Session 6

A diffusion model for ultrasound modulated light in a turbid medium

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The ability to focus light in most tissue degrades quickly with depth due to high optical scattering. Researchers have investigated using both ultrasound (US) and light synergistically to overcome this difficulty.

Ultrasound has been utilized to modulated light within tissue to create a diffusive wave at that is modulated at the US frequency. Recently, there has been interest in the modulated sidebands which reside at optical frequency plus or minus the US frequency.

This paper will put forth a model for US—light interactions in a scattering medium. We will use this model to relate the radiance in the probe beam to the radiance in the diffusive wave. We will then employ the P—1 approximation to the radiative transport equation to find the fluence and flux of the modulated wave. We will use these parameters to write a diffusion equation for the modulated wave that can be described in terms of the incoming optical power, and the US intensity and geometry. We will also illustrate the modulated sideband may be similarly approximated with its own diffusion equation.

8949-31, Session 7

Wavefront shaping of a Bessel light field enhances light sheet microscopy with scattered light

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Light sheet microscopy has seen a resurgence in fluorescence imaging as it facilitates rapid, high contrast, volumetric imaging with minimal sample exposure. Initially developed for imaging scattered light, this application of light sheet microscopy has largely been overlooked but provides an endogenous contrast mechanism which can complement fluorescence imaging and requires very little or no modification to an existing light sheet fluorescence microscope. Fluorescence and scattered light imaging differ in terms of image formation, in the former the detected light is incoherent whereas in the latter the coherence properties of the illumination source, in this case a laser, dictate the coherence of detected light, but both are dependent on the quality of the illuminating light sheet; image formation in both schemes involves the convolution of the light sheet with the specimen distribution. Separately, exciting new schemes have explored in situ wavefront shaping to overcome aberrations in the optical train and specimen.

In this paper we explore wavefront shaping for the enhancement of light sheet microscopy with scattered light. We show experimental verification of this result, demonstrating the use of propagation invariant beam types, such as the Bessel beam, to extend the field of view of a high resolution scattered light, light sheet microscope and its application to imaging of plant and mammalian super-cellular structures with sub-cellular resolution. Additionally, complementary scattering and fluorescence imaging is used to characterise the enhancement and to develop a deeper understanding of the differences of image formation between contrast mechanisms in light sheet microscopy.

8949-32, Session 7

Agile scanning using a MEMS focus control mirror in a commercial confocal microscope

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Confocal microscopes enable high-resolution imaging of thick samples including intact living tissue. Traditionally, a piezoelectric actuator or microstepper motor translates the sample mount or objective lens to change the plane of focus (i.e. z scanning) within the sample. Often, investigators acquire full x-y-z stacks in order to study features such as cell processes that do not exist in a single x-y plane. When using fluorescent markers, repeated acquisition of full x-y-z image stacks can contribute to rapid photobleaching or phototoxicity. Fast and agile focus control that is synchronized to lateral x-y scanning would allow for imaging only the relevant cross-sections within a tissue sample, thus minimizing exposure. Furthermore, an improvement in temporal resolution resulting when only relevant regions are scanned could facilitate imaging of real-time biological events.

Electrostatic microelectromechanical systems (MEMS) deformable membrane mirrors can perform agile and fast focus control. We describe here a novel MEMS mirror that is specifically designed for focus control in a scanning laser microscope. With response times less than 1 ms, our MEMS mirror allows for synchronization with the fast scan axis of a confocal fluorescence microscope to obtain unique z trajectories while the microscope's scan mirrors control imaging in x and y. Concentric control electrodes and a dynamic drive scheme allow for variable focus with concurrent management of systemic spherical aberration that is inherent in this approach to focus control. With the mirror deployed on an Olympus Fluoview FV300 we achieve more than 60 microns of focus control using a 20x 0.7 NA objective lens, and we present representative images acquired over surfaces that deviate significantly from the x-y¬ plane.

8949-33, Session 7

Full field photothermal dynamics microscopy

Guichen Tang, Fairfield Univ. (United States); Fanting Kong, Clemson Univ. (United States); Ying-Chih Chen, Hunter College (United States); Min Xu, Fairfield Univ. (United States)

We present here a novel full field pump-probe photothermal dynamics microscopy (PTDM) which uses a numerical lock-in mechanism for capturing full field photonthermal responses and is capable of imaging 2D thermal dissipation dynamics by varying the time delay between the probing and pump nano-second pulses. PTDM is built on an inverted microscope (IX60, Olympus). The pump beam is a supercontinuum pulse laser (repetition rate 20kHz) with its wavelength selected by a band pass filter. The probing beam is from a pulsed near-infrared super-luminescence diode (SLD) driven by the pump beam. The relative delay between the probing and pump pulses which can vary between 0.5?s up to 50?s (limited by the repetition rate of the pump laser) is controlled by a time delay unit. The numerical lock-in mechanism for a full field photonthermal imaging is implemented by modulating the intensity of the pump beam at a low frequency f and capturing a set of full field images at a higher frequency (at least 2f) by the CCD camera. The theory and the performance of PTDM is tested with absorbing polystyrene spheres. PTDM is ideal for monitoring temporal evolution of full field thermal response and may find interesting applications in biology and medicine. As one example, we will report the results of cancer diagnosis based on PTDM imaging nuclei contained on hematoxylin and eosin (H&E) stained prostate cancer specimens.

8949-34, Session 7

Focused beam scatterometry for deep subwavelength metrology

Thomas G. Brown, Michael J. Theisen, Stephen Head, Jonathan D. Ellis, Miguel A. Alonso, Steven R. Gillmer, Univ. of Rochester (United States)

It is known that far field scattered light requires a priori sample information in order to reconstruct nm-scale features such as are required in semiconductor metrology. We describe an approach to scatterometry that uses unconventional polarization states in the pupil of a high NA objective lens. We call this focused beam scatterometry; we will discuss the sensitivity limits to this approach and how it relates to micro-ellipsometry as well as low-NA scatterometry.

8949-35, Session 7

Phase mask optimization for 3D parallax EDF microscopy

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Extended depth-of-field (EDF) microscopy is a well investigated and very simple method to obtain projection images with an extended depth of focus (DOF) [1]. Despite its advantages of being a real-time method applicable to any microscopic mode with high lateral resolution that can be simply realized by extending a commercial microscope, the lack of z-correlation is still a problem.

Recent interesting efforts basing on the analysis of ring diameters have to deal this the tradeoff of reduced light intensity on each camera chip [2].

In this work we present a combined technique of EDF and stereo microscopy. In two pupil planes wavefronts are first delayed pixelwise by space light modulation using an SLM to obtain an extended depth of focus followed by a splitting of the ray to achieve parallax view. Two images are detected on the camera. By cross-correlation depth information is obtained while the overlay is used for image reconstruction.

For both EDF microscopy and parallax-EDF microscopy phase masks generated with the SLM are optimized by 3D PSF-engineering using CODEV simulations. Simulation results are compared to results from experiments. Finally 3D images are reconstructed for best phase masks. Time resolution of the 3D images is just limited by the camera frame rate while the spatial resolution is kept diffraction limited.

[1] E. R. Dowski and W. T. Cathey, Appl. Opt. 34, 1859 (1995)

[2] R. N. Zahreddine, R. H. Cormack, C. J. Cogswell, SPIE Proc. 8589, 858902 (2013).

8949-36, Session 8

OPC (optical phase conjugation)-assisted isotropic focusing

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Conventional optical focusing with single objective lens tends to make the spot with axial size 3-5 times worse than its transverse size. Isotropic optical focusing – the focusing of light with axial confinement that matches its lateral confinement, overcomes this limitation, and so is important for a broad range of applications. Conventionally, such focusing is achieved by overlapping the focused beams from a pair of opposite-facing microscope objective lenses. However, the exacting requirements for the alignment of the objective lenses and the method's relative intolerance to sample turbidity have significantly limited its utility.

In this paper, we present an optical phase conjugation (OPC)-assisted isotropic focusing method that can address both challenges. We exploit the time-reversal nature of OPC playback to naturally guarantee the overlap of the two focused beams even when the objective lenses are significantly misaligned (up to 140 microns transversely and 80 microns axially demonstrated). This tolerance to the misalignment is quite significant considering the precision requirement for the conventional method (~100 nm). The scattering correction capability of OPC also enabled us to accomplish isotropic focusing through thick scattering samples (demonstrated with samples of ~7 scattering mean free paths). This method can potentially improve 4Pi microscopy and 3D microstructure patterning.

8949-37, Session 8

Reducing depth-induced spherical aberration in 3D widefield fluorescence microscopy by wavefront coding using the squbic phase mask

Nurmohammed Patwary, Univ. of Memphis (United States); Ana Doblas, Univ. de València (Spain); Sharon V. King, Chrysanthe Preza, Univ. of Memphis (United States)

Imaging thick biological samples introduces spherical aberration (SA) due to refractive index (RI) mismatch between specimen and immersion medium. SA increases with the increase of either depth or RI mismatch. Therefore, it is difficult to find a static compensator for SA [1]. Different wavefront coding methods [1,3,4] have been studied to find an optimal static wavefront compensator to reduce depth- induced SA. Inspired by a recent design of a radial symmetric squared cubic (SQUBIC) phase mask that was tested for scanning confocal microscopy [1], we have modified the pupil using the SQUBIC mask to engineer the point spread function (PSF) of a wide field fluorescence microscope. In this study, simulated images of a thick test object is generated using a wavefront encoded PSF (WFE-PSF) and is restored using space invariant (SI) and depth variant (DV) expectation maximization (EM) algorithms [2] implemented in COSMOS [5]. Quantitative comparisons between restorations obtained with both the conventional and WFE-PSFs are presented. Simulations show that, in the presence of SA, the use of the SIEM algorithm and a single SQUBIC encoded WFE-PSF can yield adequate image restoration. In addition, in the presence of a large amount of SA, it is possible to get adequate results using the DVEM with fewer DV-PSFs compared to the number of clear circular aperture (CCA) PSFs that are typically required. This result implies that modification of a widefield system with the SQUBIC mask renders the system less sensitivity to depth-induced SA and suitable for imaging samples at larger optical depths.

References:

[1] G. Saavedra, I. Escobar, R. Martinez-Cuenca, E. Sanchez-Ortiga, and M. Martinez-Corral, "Reduction of spherical-aberrationa impact in microscopy by wavefront coding," OSA, vol. 17, no. 16, pp. 13810 (2009).

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[4] S. Yuan and C. Preza, "Point-spread function engineering to reduce the impact of spherical aberration on 3D computational fluorescence microscopy imaging." OSA, vol. 19, no. 23, 23298 (2011).

[5] COSMOS open source software package (http://cirl.memphis.edu/cosmos.php).

8949-38, Session 8

Pinhole array implementation of star test polarimetry

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Star Test Polarimetry is a method of inferring polarization information from a single frame point spread function [1]. We present the optical design of an imaging polarimeter that utilizes a stress engineered optical element to image the polarization states of scattered light collected by a lens across a given field. In our scheme, an intermediate image is projected to a pinhole array and the relay system is designed to produce a polarization dependent shape of the point spread function. When incorporated as a relay system in a microscope, the pinhole array can also function in a confocal arrangement, simultaneously providing depth slices and polarization information. The design and results presented are stage one of the optical layout of what we call a star test imaging polarimeter.

8949-39, Session 8

Implementation of PSF engineering in high-resolution 3D microscopy imaging with a LCOS (reflective) SLM

Sharon V. King, Univ. of Memphis (United States); Ana Doblas, Univ. de València (Spain); Nurmohammed Patwary, Univ. of Memphis (United States); Genaro Saavedra, Martínez-Corral Manuel, Univ. de València (Spain); Chrysanthe Preza, Univ. of Memphis (United States)

Wavefront coding techniques are used to manipulate phase and amplitude properties of the optical wavefront at the imaging system exit pupil and to create unique engineered point spread functions (PSFs) [1]. Implementation of PSF engineering with a liquid crystal on silicon (LCoS) spatial light modulator (SLM) provides a means to validate WFC phase masks designs and parameters with a programmable diffractive element [2]. We implemented PSF engineering in a Zeiss AxioImager modified with a dual camera port and a LCoS SLM. We effectively measure the PSF by captured images of unresolved fluorescent beads with a high NA objective. We present measured WFC PSFs implemented with a LCoS SLM and compare them to simulated PSFs through analysis of their effect on the properties of microscope imaging system. Previous work demonstrated that simulated intensity PSFs encoded with a generalized cubic phase mask (GCPM) are invariant to either spherical aberration or misfocus dependent on mask design parameter selection [3]. Additionally, a squared cubic phase mask (SQUBIC) has been shown to produce an invariant focal spot in depth-scanning of thick samples for confocal scanning microscopy [4, 5]. Our experimentally acquired PSFs show the same intensity distribution as simulation for the GCPM phase mask, the SQUBIC-mask and the well-known and characterized cubic-phase mask (CPM), first applied to high NA microscopy by Arnison et al. [6], for extending depth of field. These measurements provide experimental validation of new WFC masks and demonstrate the use of the LCoS SLM as a WFC design tool.

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8949-40, Session 8

Investigation of the squbic phase mask design for depth-invariant widefield microscopy point-spread function engineering

Ana Doblas, Univ. de València (Spain); Sharon V. King, Nurhmohammed Patwary, Univ. of Memphis (United States); Genaro Saavedra, Manuel Martinez-Corral, Univ. de València (Spain); Chrysanthe Preza, Univ. of Memphis (United States)

In this contribution the complex transmittance at the objective exit pupil [1,2] is modified with the insertion of a squared cubic phase mask (SQUBIC). The transmittance of this mask depends on a single parameter related with the maximum value of the phase over the pupil. The SQUBIC -based design is purpose-built for reducing the SA impact in imaging systems. In previous work [3] the computed SQUBIC-PSFs with SA is presented and the invariance of the focal spot in the depth-scanning processing of thick samples for confocal scanning microscopy is successfully demonstrated for a specific SQUBIC pupil function design. In this study, we find theoretically the transformation law for the width of the axial PSF under SA for any pupil function design [4]. This formulation provides us a powerful technique for the optimal design of the SQUBIC mask. By evaluating the axial PSF for different SQUBIC-mask designs, it is easy to show that the high-er the value of the SQUBIC-mask design parameter, the less sensitive the imaging system is to SA. Furthermore, from this reasoning, we created a merit function, which we call Rayleigh pa-rameter, R, such that any pupil mask that provides $R > 0$ will increase the robustness of the PSF in the presence of SA in comparison to the conventional system. Our results also show that SQUBIC-PSFs are less invariant in the presence of SA in comparison with other CPM-designs [5].

8949-41, Session 9

Implementation of aberration correction in an adaptive optics STED microscope

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High resolution microscopy relies on the use of high quality optics with the goal of obtaining diffraction-limited operation, working at the physical limits imposed by the wavelength of the light. Yet in many cases this goal is not achieved as aberrations, distortions in the optical wavefront, blur the focus and reduce the resolution of the system. Aberrations can arise from imperfections in the optics, but are often introduced by the specimen, particularly when imaging thick specimens. Adaptive optics systems enable the dynamic correction of aberrations through the reconfiguration of an adaptive optical element, for example a deformable mirror or liquid crystal spatial light modulator. Stimulated emission

depletion (STED) microscopes enhance the resolution of a fluorescence microscope to the tens of nanometres level. These microscopes are even more sensitive to specimen induced aberrations. An adaptive STED microscope was recently introduced, in which a liquid crystal spatial light modulator was used to implement both phase masks for focal shaping and to correct aberrations. We report on new adaptive STED microscope systems using both a deformable mirror and a spatial light modulator to provide enhanced correction capabilities. In particular, we explain the methods required to optimise performance of the microscope for correction of specimen induced aberrations.

8949-42, Session 9

Customized profile lens based linear response on-axis optical scanners for 3D laser scanning microscopy

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The use of steered optical beam systems is common in 3D laser scanning microscopy systems, e.g. confocal reflectance microscopy, multi-photon microscopy, etc. Currently, reflection mirrors mounted on polygons and glavos are the most widely used beam steering techniques. However, these approaches are off-axis solutions, which increase the size, the error, and the complexity of the overall system. Some other on-axis approaches have been demonstrated in the past. But from an engineering standpoint, these approaches pose difficulties, which limit their use in imaging application. Previously, we presented an on-axis linear-response linear-motion optical scanner. While the linear design is highly desired for engineering consideration, it was still lacking the scanning speed required for imaging applications. We here present a customized profile lens (CPL), tailored for high speed performance while maintaining the advantages of a linear response on-axis optical scanner. The feasibility of the design's functionality was first evaluated analytically using Matlab and ray-tracing techniques. The device was then built and tested experimentally on an optical bench. The test results show agreement with our simulations, demonstrate precise linear response and fast scanning speed, and revealed video frame rate scanning ability. A second design more suitable for mass production of the CPL is presented. The implementation of the CPLs in laser scanning systems is promising in improving the current 3D laser scanning microscopy systems, as well as other systems unitizing high speed laser scanning technique.

8949-43, Session 9

Label-free molecular imaging

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Optical microscopy technology has achieved great improvements in the 20th century. The detection limit has reached about twenty nanometers (with near-field optics, STED, PALM and STORM). But in the application areas such as life science, medical science, clinical treatment and especially in vivo dynamic measurement, mutual restrictions still exist between numeric aperture/magnification and working distance, fluorescent dependent, and between resolution and frame rate/field size, etc.

This paper explores a high spectral scanning super-resolution molecules imaging in label free based on the white light interferometry. The detection resolution in axis was approximate to 1nm in single molecular layer level and dynamic measuring range of thickness reaches to 10μm. The spectrum-shifting algorithm is developed for robust restructure of images when the pixels are overlapped. Micro-biochip with protein binding and DNA amplification could be detected by using this spectral scanning super-resolution molecules imaging in label free. This method has several advantages as following: Firstly, the decoding and detecting steps are combined into one step. It makes tests faster and easier. Secondly, we used thickness-coded, minimized chips instead of a large microarray chip to carry the probes. This accelerates the interaction of the biomolecules. Thirdly, since only one kind of probes are attached to our thickness-coded, minimized chip, users can only pick out the probes they are interested in for a test without wasting unnecessary probes and chips.

8949-44, Session 9

Hyperspectral multipoint confocal microscope

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Spectral analysis is an important tool in microscopy for example to distinguish signals from multiple endogenous and exogenous fluorophores. When imaging dynamic or fragile samples, such as in-vivo specimens, the utility of spectral resolution has to be balanced with the requirements for fast capture, high-sensitivity and minimal sample irradiation. Traditional confocal microscopes operating with a single aperture and using a camera or photomultiplier (PMT) as a single pixel readout are limited by the time taken to scan a raster, the relatively low quantum efficiencies of currently available PMTs, and fluorophore saturation.

A solution involves scanning with multiple pinholes together with the use of a high-sensitivity imaging camera as readout. Spectral resolution in such multipoint confocal microscopes is often obtained by multiple exposure, using different color filters. This technique is easy to implement, but is slow and an inefficient use of the fluorescent signal.

We present a 15 channel spectral microscopy system based on a Prairie Technologies Swept Field Confocal microscope with an Amici prism as the dispersive element. Amici prisms provide wavelength separation without deflecting the mean direction of the beam and can therefore be easily added to existing designs.

Our prism can be translated in and out of the beam without the need for further calibration to switch from a standard confocal to a hyperspectral system. By scanning several pinholes in parallel this system can achieve high capture speeds. The high quantum efficiency of the EMCCD and the low loss of the prism provide high sensitivity and low noise.

8949-45, Session 9

In vivo stepwise multi-photon activation fluorescence imaging of melanin in human skin

Zhenhua Lai, Zetong Gu, Saleh Abbas, Charles A. DiMarzio, Northeastern Univ. (United States)

Previous research has shown that the stepwise multi-photon activated fluorescence (SMPAF) of melanin is a low cost and reliable method of detecting melanin because the activation and excitation can be a

continuous-wave (CW) mode near infrared (NIR) laser. In this study, in vivo SMPAF images of melanin in human are compared with conventional multi-photon fluorescence microscopy (MPFM) images and confocal reflectance microscopy (CRM) images to prove the effectiveness of SMPAF in melanin detection. SMPAF images add specificity for melanin detection than MPFM images and CRM images. SMPAF images also demonstrate potential to increase sensitivity for detecting small size melanin granules. Melanin SMPAF is a promising technology to enable early detection of melanoma for dermatologists.

8949-46, Session 9

Noise removal techniques for microscope images: a comparison

Carol J. Cogswell, Ramzi N. Zahreddine, Robert H. Cormack, Univ. of Colorado at Boulder (United States)

No Abstract Available

8949-47, Session 10

High-resolution quantitative phase imaging with orientation-independent differential interference contrast (OI-DIC) microscope

Michael I. Shribak, Marine Biological Lab. (United States)

Recently developed orientation-independent differential interference contrast (OI-DIC) microscope allows the bias to be modulated and shear directions to be switched rapidly without any mechanically rotating the specimen or the prisms. However the practical application of OI-DIC technique requires significant modification of a regular microscope. We report about new compact OI-DIC assembly, which fits into existing slot of research grade Olympus microscopes, and which does not require a microscope modification. It can also be used with available high-NA objective lenses providing the high-resolution imaging. The assembly consists of two standard DIC prisms with liquid crystal cell in between. Another liquid crystal cell is employed for modulating a bias. Within a second the microscope captures a sets of raw DIC images at the orthogonal shear directions and different biases. We describe principles of computing the quantitative optical phase and phase gradient images. Newly developed OI-DIC technique can be combined with other imaging modalities such as fluorescence and polarization.

8949-48, Session 10

Phase unwrapping in interference microscopy: how to make time-sequential data meaningful

Goldie L. Goldstein, Univ. of Arizona (United States); Katherine Creath, 4D Technology (United States) and Optineering (United States)

Interference Microscopy techniques often need phase unwrapping methods to create meaningful data that is biologically relevant. Furthermore, time-sequential data needs to be unwrapped in such a way that it is self-consistent with minimal 2π phase jumps between frames. This paper will focus on phase unwrapping methods that show promise for being relevant in this field and introduce some new techniques to ensure consistent phase unwrapping in the time domain.

Several well-documented phase unwrapping methods will be implemented to test the accuracy of the unwrapped phase of several biological datasets. A method will be introduced that can rate the relative accuracy between unwrapping methods. This will serve as a baseline for choosing an algorithm to proceed forward into the time-domain.

When using a time-series of interference microscopy measurements to do science, the unwrapped phase cannot be ambiguous. If the "best" frame within a sequence of data can be isolated, then the rest of the measurements from the dataset can be forced to unwrap in the same manner as the baseline frame. This allows the ability to observe quantifiable metrics, such as integrated optical volume over local areas, without the concern that phase unwrapping errors are causing erroneous data.

8949-49, Session 10

Spectral-domain interferometry for quantitative DIC microscopy

Yizheng Zhu, Chengshuai Li, Virginia Polytechnic Institute and State Univ. (United States)

Optical phase is perhaps the most important intrinsic contrast mechanism for stain-free imaging of biological samples. Primary examples of this technique include phase contrast microscopy and differential interference contrast microscopy. However, the conventional implementation of these techniques are qualitative in that optical phase of the sample is converted into intensity change nonlinearly and is hence hard to be obtained directly. This is further compounded by the intrinsic attenuation variation across the sample. In recent years, various quantitative phase microscopy techniques have been developed capable of separating and quantifying sample phase and amplitude. Examples include digital holography, phase-shifting phase contrast/DIC microscopy, and optical coherence microscopy, among others. Owing to their inteferometric approach, these techniques are capable of sensitive phase measurement at nanometer/sub-nanometer scale.

In this presentation, we will introduce spectral-domain DIC (SD-DIC) microscopy and its most recent progress. SD-DIC microscopy is a novel quantitative phase imaging technique that combines the high sensitivity of spectral-domain low coherence interferometry with the high stability of DIC configuration to achieve high resolution decoupling and quantification of optical phase gradient and intensity of a sample. The key element in the system is a phase retarder that introduces a large optical pathlength difference between the o- and e- waves, facilitating sensitive phase demodulation. Recent results of using SD-DIC for reconstructing two-dimensional phase topography and detecting metallic nanoparticles will be discussed.

8949-50, Session 10

Portable interferometry

Natan T. Shaked, Pinhas Girshovitz, Tel Aviv Univ. (Israel)

We present our late advance in the development of compact, highly portable and inexpensive interferometric modules, which do not require the strict stability and the highly-coherent illumination that are usually required for conventional interferometric or holographic setups. The proposed modules use off-the-shelf optical elements and they do not require extensive optical knowledge or meticulous alignment prior to every experiment. Still, due to their common-path, low-coherence design, they can measure the quantitative optical thickness maps of thin samples with stabilities of down to 0.2 nm. The proposed designs are provided with software that can process the phase profile (including phase unwrapping) in real time. These capabilities make interferometric imaging more accessible for non-expert users, including biologists and clinicians.

8949-51, Session 10

Reflective interferometric system combining low-coherence spectral-domain phase microscopy and wide field holography for characterization of thin samples

Reut Friedman, Natan T. Shaked, Tel Aviv Univ. (Israel)

We introduce an integrated system combining low-coherence spectral-domain phase microscopy (SDPM) together with a compact, simple-to-align, wide-field high-coherence interferometer (off-axis TAU module) for accurate quantitative phase measurements. The proposed compact system is capable of capturing an off-axis, wide-field interference in the time domain simultaneously with a single-point interference in the spectral domain. The integrated system can obtain both quantitative phase of transparent samples, requiring a reflective surface at the back of the sample, or profiling of reflective samples. Since there are no moving elements in the system, it is capable of measuring static and dynamic samples, while time resolution is limited only by the frame rate of the detectors (a camera and a compact spectrometer). Both interferometers are in common-path geometry, resulting in high signal to noise ratio and nanometer-scale stability. The combined system is ideal for characterization of static or dynamic samples containing both wide areas of interest that can be acquired by the off-axis TAU module, and specific spots of interest requiring fine measurement that can be acquired by SDPM. Several possible applications of the integrated system are presented, including various optical metrology experiments.

8949-52, Session 11

Speckle-free sub-diffraction resolution quantitative phase imaging via structured illumination

Shwetadwip Chowdhury, Joseph Izatt, Duke Univ. (United States)

In the biological sciences, quantitative phase maps of cells allow detailed visualization of cellular structure and composition with minimal sample preparation and can accurately capture even minute cellular fluctuations via phase contrast. In cases where conventional preparation techniques, such as fixation, staining, or fluorescent tagging, may affect cellular functions and limit biological insight, quantitative phase microscopy (QPM) offers an important alternative to image transparent samples with no exogenous contrast agent and with high contrast and visibility. In other cases, such as in determining cellular path lengths or refractive index, QPM is one of the few available options. However, QPM is a coherent imaging technique and thus suffers from speckle noise artifacts as well as an intrinsic resolution loss when compared to incoherent imaging techniques. To be comparable to these incoherent imaging techniques, there is a direct need to adapt QPM for speckle-free imaging as well as to extend QPM's imaging resolution to sub-diffraction levels. Here, we introduce an extension to structured illumination microscopy that allows for sub-diffraction resolution, speckle-free, quantitative phase imaging of transparent samples. We experimentally demonstrate our system's quantitative capabilities by showing phase maps of calibration phase samples, and show that the phase maps accurately match the phase profiles of known samples with high fidelity. We also demonstrate our system's capabilities to image biologically relevant samples by showing high contrast, speckle-free, quantitative phase images of mesenchymal stem cells at sub-diffraction resolutions.

8949-53, Session 11

Membrane-substrate separation distance assessed by normalized total internal reflection fluorescence microscopy.

Marcelina Cardoso Dos Santos, Rodolphe Jaffiol, Vézy Cyrille, Univ. de Technologie Troyes (France)

Total Internal Reflection was applied for the first time in cellular imaging by E.J. Ambrose during the 50's. Many years after, D. Axelrod has greatly helped popularize this technique in biology. As a consequence of the recent progress of optical tools (high numerical aperture objectives NA>1.4, high sensitive detectors like EMCCD...) Total Internal Reflection Fluorescence Microscopy is becoming a standard technique to study the plasma membrane of living cells.

TIRFM use an evanescent wave to reduce the excitation depth at the vicinity of the glass-water interface, typically 100 nm. We developed a prismless device (i.e. the same objective is used to create the evanescent wave and to collect the fluorescent signal) equipped with a motorized mirror mount to adjust the lateral position of the incoming laser beam on the entrance pupil of the objective. It allows to switch easily between TIRFM and conventional wide-field fluorescence imaging. In this work, we propose a new method to extract quantitative information about the distance between the membrane and the surface with a nanoscale resolution. To achieve this, TIRFM images need to be normalized by usual wide-field fluorescent images [1]. This technique is particularly interesting to explore the adhesion of Giant Unilamellar Vesicles on various chemically functionalized glass substrates.

[1] Axial nanoscale localization by normalized total internal reflection fluorescence imaging, M. Cardoso Dos Santos et al., submitted in Optics Letters.

8949-54, Session 11

Sub-diffraction-limited imaging of fluorescent protein expressed in living cells by saturated excitation (SAX) microscopy

Masahito Yamanaka, Kenta Saito, Nicholas I. Smith, Satoshi Kawata, Takeharu Nagai, Katsumasa Fujita, Osaka Univ. (Japan)

Saturated excitation (SAX) microscopy exploits the nonlinear relationship between excitation and fluorescence intensity induced by the saturation of the population of the excited state to improve the spatial resolution in three dimensions. Here, we report for the first time the SAX of fluorescent proteins for sub-diffraction-limited imaging of living cells. To confirm the capability of SAX of fluorescent proteins, we have measured the fluorescence intensity from Venus and EGFP under various excitation intensities, and the nonlinear relationships between the excitation and the fluorescence, which contributes to the improvement of the spatial resolution, has been confirmed. Mitochondria of a Hela cell were labeled by Venus and excited with a 532-nm continuous-wave (CW) laser. We also observed living HeLa cells expressing a green fluorescent protein (EGFP) in the Golgi apparatus by using a 488-nm CW laser for the excitation. The results show that both Venus and EGFP provide sufficient nonlinear fluorescence signals under the intracellular environment to perform sub-diffraction-limited imaging in SAX microscopy. Liner deconvolution is also applied to the images obtained by SAX microscopy, and it is confirmed that the combination of linear deconvolution and the expansion of the optical transfer function by SAX is effective in further enhancing the contrast of small intracellular structures in the SAX images.

Conference 8950: Single Molecule Spectroscopy and Superresolution Imaging VII

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8950 Single Molecule Spectroscopy and Superresolution Imaging VII

8950-1, Session 1

Mechanical manipulation of the electronic states of a single molecule by scanning force microscopy

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We have utilized an AFM tip to apply compressive stress on individual functionalized chromophores, spread on a mica surface. Simultaneously, we followed the impact of the force on the electronic transition energies of the molecule by confocal fluorescence microscopy. Our dye consists of a terrylene-diimide (TDI) core decorated with four bulky perylene-diimide (PDI) arms. Quantum chemical calculations have predicted a significant twisting of the TDI core. Depending on this twisting and the orientation of the side-groups with respect to the TDI core several conformations of the overall molecule exists, accompanied by different electronic transition energies.

Our goal was to mechanically manipulate the conformational state of a single chromophore while probing its photophysical properties. The experimental setup consisted of a home built confocal inverted microscope optimized for single molecule detection in conjunction with a commercial atomic force microscope. For vibrational isolation the whole setup was placed into a closed box, also allowing for sufficient temperature stabilization. Especially the latter turned out to be crucial for our experiments to minimize thermally induced drifts. A thorough alignment ensured that both microscopes simultaneously probed the same areas within 20 nm, far below the optical resolution of 300nm.

By increasing the force on single molecules in the repulsive interaction regime, reversible spectral shifts as well as transitions into metastable states were observed. The experimentally obtained range of the force-induced spectral shifts has been reproduced by quantum chemical calculations of the transition energies of different molecular conformations.

8950-2, Session 1

Enhanced 3D localization of individual RNA transcripts via astigmatic imaging

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The ability to detect and count individual messenger RNA molecules in single cells is a powerful tool to explore gene expression networks. The primary technique for RNA counting is single molecule fluorescence in situ hybridization (smFISH). smFISH has been successful in elucidating many gene regulatory processes but is often plagued by the poor z-localization inherent with wide-field illumination. This lack of z resolution can lead to overlapping spots of mRNA and less information regarding as to what compartment of the cell the mRNA is located (e.g. nucleus, cytoplasm, or membrane-associated). Here we have applied astigmatic imaging to smFISH as a means to improve the z-localization. The instrument we have developed is capable of high throughput automated multicolor smFISH acquisition with or without astigmatic imaging. In addition we have incorporated an existing graphics processing unit (GPU) based spot fitting algorithm into our custom smFISH analysis software. This instrument enables single RNA counting in large cells and tissues where high precision localization is important in all three dimensions. We apply this astigmatic smFISH microscope to imaging of differentiated mesenchymal stem cells and show detection of RNA transcripts through a range of 20 micrometers with a localization precision of 200 nanometers in the z dimension. We observe significantly better localization in z, which enables better resolution of co-localized spots and clusters of RNA such as at nuclear transcription sites.

8950-3, Session 1

Spectroscopic and transport measurements of single molecules in solution using an electrokinetic trap *(Invited Paper)*

Quan Wang, William Esco Moerner, Stanford Univ. (United States)

Diffusion generally limits the observation window of single molecules in solution to milliseconds and prevents quantitative determination of spectroscopic and transport properties molecule-by-molecule. The Anti-Brownian electrokinetic (ABEL) trap enables prolonged (>1 seconds) observation of single molecules in solution by approximately canceling Brownian motion through active feedback. The amount of information that can be extracted from each molecule in solution is thus boosted by three orders of magnitude. We describe our recent advances in extending the ABEL trap to conduct both spectroscopic and transport measurements of single trapped molecules.

Using simultaneously-recorded spectroscopic parameters (brightness, excited-state lifetime and emission spectrum), we study the photophysical properties of single copies of Atto647N and Atto633, two of the most popular fluorescent labels used in single-molecule spectroscopy, in solution. Interestingly, both dyes occasionally show inter-conversions between three distinct fluorescent states. We characterize these states in detail.

Using statistically learned transport coefficients (diffusion coefficient and electrokinetic mobility), we demonstrate real-time monitoring of single bi-molecular binding/unbinding interactions in solution with 50ms time resolution. We characterize the kinetics and extract the free energy of the interaction for the first time.

8950-4, Session 1

Multi-pulse two-color detection of exocytotic mucin release and swelling using Acridine Orange

Dmytro Shumilov, Joseph D. Kimball, Texas Christian Univ. (United States); Rafal Fudala, Univ. of North Texas Health Science Ctr. at Fort Worth (United States); Irina Akopova, Univ. of North Texas Health Science Ctr. at Fort Worth (United States); Ignacy Gryczynski, Julian Borejdo, Univ. of North Texas Health Science Ctr. at Fort Worth (United States); Zygmunt Gryczynski, Texas Christian Univ. (United States); Ryszard Grygorczyk, Univ. de Montréal (Canada)

Mucus secretion is the first-line of defense against the barrage of irritants inhaled into human lungs and abnormally thick and viscous mucus results in many respiratory diseases such as cystic fibrosis (CF), asthma, and chronic obstructive pulmonary disease. Investigation of processes underlying mucus pathology is hampered, in part, by lack of appropriate experimental tools for labeling and studying mucin granule secretion from live cells with high sensitivity and temporal resolution. We have been utilizing original spectroscopic properties of acridine orange (AO) for studying granule release and mucin swelling. In this report, we are presenting a novel approach based on multiple-pulse two-color excitation and two-color observation which allows us to extend the detection range over an order of magnitude in variable concentration of AO.

Using controllable bursts of high repetition pulses spaced at lower repetition packets we are able to highly increase and modulate the fluorescence signal of long-lived aggregates over the monomers, greatly increasing the dynamic range for detecting AO dilution and mucin expansion. A ratiometric detection allows time-resolved (FLIM-type) monitoring of exocytotic process with a video rate over 10 frame per second.

8950-5, Session 2

Image classification based on high-content fluorescence correlation spectroscopy (HCS-FCS) measurements *(Invited Paper)*

Winfried Wiegraebe, Qingfeng E. Yu, Christopher J. Wood, Jeffrey J. Lange, Lucinda E. Maddera, Stowers Institute for Medical Research (United States)

We developed tools for high-content fluorescence correlation spectroscopy (FCS).

We determined for the whole yeast genome the diffusion properties and local concentrations of its proteins[1]. We extended this automated approach to fluorescence cross-correlation spectroscopy (FCCS)[2].

We acquired a transmitted light image of each cell we measured. We used these images to include only data from healthy cells into our analysis. We used machinelearning tools to judge the state of a cell. In a first approach, we used human experts to create training sets.

Surprisingly, human experts showed only a classification accuracy of about 80% in repeat experiments. Therefore, we decided to use FCS correlation curves to create training sets. We assumed that one specific protein would have similar diffusion properties in all healthy cells. We collected sets of FCS curves from six different proteins. We averaged all correlation curves for a given protein to construct a ‘typical’ measurement. Then, we ranked all individual cells based on the distance between their individual correlation curves from the ‘typical’ curve. The larger this distance was, the lower we put the likelihood that the measurement came from a healthy cell. We used this information to construct a new training set to classify the segmented cells.

1. Wood, C., et al., Fluorescence correlation spectroscopy as tool for high-content screening in yeast (HCS-FCS). Proceedings of the SPIE, 2011.

2. Lange, J.J., et al., Correction of bleaching artifacts in high content fluorescence correlation spectroscopy (HCS-FCS) data. 2013: p. 859006?859006

8950-6, Session 2

Determination of protein concentration on substrates using fluorescence fluctuation microscopy

Richard De Mets, Irène Wang, Univ. Joseph Fourier (France); Joseph Gallagher, Université Joseph Fourier (France); Olivier Destaing, Institut Albert Bonniot (France); Martial Balland, Antoine Delon, Univ. Joseph Fourier (France)

The preparation of substrates, chemically and mechanically controlled at the micrometer scale, is very important for the study of adherent living cells and mechanisms such as migration, differentiation, survival and cell proliferation. Indeed, the cells are able to exchange information with their substrate, with the effect of modifying some physiological parameters, such as the force exerted by the cells, or their spreading.

Microfluidic techniques now make it possible to achieve patterned substrates, that is to say surfaces (gels, polymers, glass, etc..) on which grafted molecules interact with cells via membrane receptors. The homogeneity and reproducibility of the molecular concentration on these patterns is a critical parameter that is, paradoxically, poorly controlled and even less quantified.

We have used a technique belonging to the family of Fluorescence Fluctuation Microscopy (FFM), to measure the surface concentration of molecules anchored on substrates. The autocorrelation of the confocal image obtained by scanning the sample is directly related to the average number of molecules simultaneously illuminated by the laser beam and therefore to their concentration. We have studied the relation between the number density of molecules (proteins) initially present in the solution deposited on the surface and the final surface concentration of these proteins. We suggest additional methods to account for surface inhomogeneities and estimate the actual concentration, together with control parameters of the procedure.

8950-7, Session 2

Single-molecule fluorescence spectroscopy in nanochannels

Siddharth Ghosh, Jan Thiart, Jörg Enderlein, Georg-August-Univ. Göttingen (Germany)

Single-molecule fluorescence spectroscopy of molecules freely diffusing in solution has become an important research tool in biophysics and physical chemistry. One of the major limitations of these experiments is the finite residence time of a few micros- to milliseconds of a molecule within the detection volume, which has typically a size of ca. one femtoliter. Here, we present an experimental approach that combines three ideas for indefinitely extending the residence time of a single molecule within the laser focus of a confocal fluorescence microscope. We use nanochannels of ca. 100 nm diameter to restrict the molecular motion along the optical axis and along one lateral axis, and we use dual-focus fluorescence detection in combination with an Anti Brownian Electrophoretic or ABEL trap to actively trap the molecule along the second lateral dimension. By passivating the nanochannel surface with a lipid monolayer, unwanted adsorption of molecules to the nanochannels’ walls is suppressed.

8950-8, Session 2

With photon patterns towards species selective microscopy

Felix Koberling, Volker Buschmann, Benedikt Kraemer, Steffen Ruettinger, Marcelle Koenig, Sebastian Tannert, Matthias Patting, Rainer Erdmann, PicoQuant GmbH (Germany)

Fluorescence Correlation Spectroscopy is nowadays a standard tool in biophysics and more and more used also in complex environments, like in cell biology and mutilabel applications. Common problems which complicate these experiments, like detector afterpulsing and spectral crosstalk, have recently be overcome by looking at the nanosecond arrival time of the detected photons after pulsed excitation. By comparing the nanosecond arrival time of every photon with reference decay patterns it is possible to identify artifact signals and to distinguish

and separate photons coming from species with different emission lifetime properties.

We will show current results for absolute concentrations measurements of diffusing proteins in live cells as well as dual colour FCCS binding studies. Especially in dual colour applications when two pulsed lasers are not available the decay pattern analysis allows quantitatively to separate the pulsed laser excited fluorescence from the cw excited one to overcome spectral bleedthrough problems.

In our latest work we generalize the concept of single photon classification beyond one-dimensional patterns and correlation spectroscopy in diffusion. A simplified approach for photon ensembles is a decay pattern matching analysis describing the photon ensemble decay with a linear combination of reference decays which can be highly multi-exponential and do not have to be described itself mathematically. We will show this approach to be a powerful alternative to multi-exponential decay fitting based FLIM and consider it even applicable to burst classification in single molecule experiments.

8950-9, Session 2

Development and application of two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS)

Kunihiko Ishii, Tahei Tahara, RIKEN (Japan)

Fluorescence correlation spectroscopy (FCS) is a unique tool for investigating microsecond dynamics of complex biological molecules in equilibrium. However, application of FCS in the study of molecular dynamics has been limited, owing to the difficulty in resolving the dynamics of multiple species in the sample. To solve this problem, we have developed a new method that combines FCS and time-correlated single-photon counting (TCPSC). In this method, which we name two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS), we analyze the correlation of fluorescence photon pairs referring to the fluorescence lifetime. We first obtain the correlations of the photon pairs with respect to the excitation-emission delay times in the form of a 2D map. Then, the 2D map is converted to the correlations between different species based on their fluorescence lifetimes using inverse Laplace transformation. Any prior information about the fluorescence decay curves of the constituent species is not necessary in 2D FLCS, in contrast to the existing method of TCSPC-FCS data analysis, i.e., fluorescence lifetime correlation spectroscopy (FLCS). We applied 2D FLCS to the dynamics of a DNA hairpin labeled with a FRET pair. By constructing the 2D lifetime correlation maps of the FRET donor, the equilibrium dynamics between the open and the closed forms of the DNA hairpin was clearly observed as the appearance of the cross peaks between the corresponding fluorescence lifetimes. The present study clearly shows that 2D FLCS can disclose equilibrium structural dynamics of biological molecules with microsecond time resolution.

8950-10, Session 2

Quantifying aqueous membrane protein interactions using fluorescence correlation spectroscopy

Sonny Ly, Feliza A. Bourguet, Nicholas O. Fischer, Matthew A. Coleman, Ted A. Laurence, Lawrence Livermore National Lab. (United States)

Interactions involving membrane proteins are involved in and control a multitude of cellular processes, including signal transduction, energy production and conversion, cell adhesion, and foreign molecule identification. More than half of all pharmaceutical drugs target membrane proteins, further illustrating their importance in human health. Quantitative measurements of binding affinities of protein-protein interactions are difficult when they involve membrane-bound proteins, despite the central importance of such measurements. Recently, nanolipoprotein (NLP) technology has been used to solubilize membrane proteins in a native-like lipid environment while maintaining the protein's functionality. This success has opened a realm of possibilities in analyzing membrane protein dynamics in solution. Here we demonstrate that fluorescence correlation spectroscopy (FCS) is a rapid and effective method to characterize pathogen-related interactions between soluble and membrane embedded proteins supported within NLPs. Using FCS, we measured a binding affinity of 21 nM between EGFP-labeled protein LcrV and the membrane-bound protein YopB inserted into a NLP scaffold. The combination of FCS and NLP technology provides a powerful approach to accurately measure equilibrium constants that is difficult by other means. This approach can be applied to a wide a range of ligand-receptor interactions between membrane bound and soluble proteins.

8950-11, Session 3

Single molecule fluorescence saturation spectroscopy

Rodolphe Jaffiol, Pascale Winckler, Univ. de Technologie Troyes (France)

Saturation spectroscopy is a relevant method to investigate photophysical parameters of single fluorescent molecules. This experimental technique appears especially to be one of the most promising ways to decipher in plasmonics the interactions between dye molecules and metallic nanostructures, for example to quantify the enhancement process [1]. Nevertheless, the impact of a gradual increase, over a broad range, of the laser excitation on the intramolecular dynamics is not completely understood, particularly concerning their fluorescence emission (the so-called brightness). Thus, we propose a comprehensive theoretical and experimental study to interpret the unexpected evolution of the brightness with the laser power taking into account the cascade absorption of two and three photons [2]. Furthermore, we highlight the key role played by the confocal observation volume in fluorescence saturation spectroscopy of single molecule in solution.

[1]. J. Wenger et al., J. Phys. Chem. C 2007, 111, 11469-11474.

[2]. P. Winckler and R. Jaffiol, Anal. Chem. 2013, 85, 4735-4744.

8950-12, Session 3

Accelerated single photon emission from dye molecule driven gold nanoparticle dimers assembled on DNA

Vincent Maillard, CNRS (France) and École Supérieure de Physique et de Chimie Industrielles (France); Mickael P. Busson, CNRS (France); Brice Rolly, Aix-Marseille Univ. (France); Petru Ghenuche, CNRS (France); Brian D. Stout, Aix-Marseille Univ. (France); Nicolas Bonod, Jerome Wenger, Sebastien Bidault, CNRS (France)

Because of homogeneous broadening effects, single organic molecules exhibit weak absorption cross-sections at room temperature even though they feature large dipolar transition moments for their electronic excited states. We recently demonstrated, by a conjunction of time-resolved luminescence and fluorescence correlation spectroscopy, that gold nanoparticle (AuNP) dimers can be used to enhance reproducibly the excitation cross-sections and decay rates of organic dyes by more than one order of magnitude (M. P. Busson et al, Angew. Chem. Int. Ed. 51, 11083, 2012). We use 40 nm diameter AuNPs linked by a single DNA strand as short as 10 nm and electrophoretic purification to obtain a stable suspension of dimers (M. P. Busson et al, Nano Lett. 11, 5060, 2011). By controlling the number of DNA linkers, we ensure that only

one quantum emitter is attached per nanostructure, allowing single photon emission with lifetimes as short as 30 ps (M. P. Busson et al, Nat. Commun. 3, 962, 2012). These nanostructures behave as the optical equivalent of a dipolar antenna driven by a single photon source. In order to optimize the quantum yield and excitation probability of these emitters, we increase the size of the AuNPs and the scattering cross-sections of the antenna in order to reach an average 44 times enhancement of the fluorescence count rate with picosecond lifetimes. These values correspond to unprecedented dipolar transition moments of isolated quantum emitters at room temperature.

8950-13, Session 3

Versatile pulsed 560-nm laser source for FCS and FLCS

Thomas Schoenau, Susanne Trautmann, Kristian Lauritsen, Haertel Romano, Klemme Dietmar, Rainer Erdmann, PicoQuant GmbH (Germany)

We present a 559 nm picosecond pulsed laser, based on a fiber amplified and frequency doubled gain-switched laser diode. Gain-switched laser diodes give the opportunity to trigger single pulses from an arbitrary electrical signal source. Optical pulses, generated in an 1118 nm DFB gain-switched laser diode with an energy of a few picojoules are amplified in a single-stage Yb-doped fiber amplifier. For efficient amplification, a fiber with Al-codoping was selected to increase the emission cross section of the amplifier around 1120 nm. Furthermore, suppression of amplified spontaneous emission (ASE) and parasitic lasing at shorter wavelengths is investigated. The output of the amplifier is spliced to a fiber coupled waveguide crystal made of periodically poled KTP (potassium titanyl phosphate) for single pass SHG to 559 nm.

This freely triggerable laser source can operate in a wide range of repetition frequencies from 1 MHz up to 80 MHz which makes it easy to adapt the pulse period to different fluorescence lifetimes. Synchronization to other lasers or scanning devices is also possible as well as burst operation. Full characterization of spectral and pulse properties will be presented.

The wavelength around 560 nm is appropriate for a wide range of applications, especially in the life-sciences where state of the art optimal red fluorescent proteins excited at about 560 nm are essential. A pulsed 559 nm laser will enable the long sought Fluorescence Lifetime Correlation Spectroscopy (FLCS) using eGFP and mCherry to eliminate fluorescence background and spectral cross-talk from molecular interaction measurements. Implemented in a confocal microscope, like the MT 200 (PicoQuant), this laser becomes a versatile tool for the life sciences.

8950-14, Session 4

Multiplex fluorescence marker quantification with spectrally-resolved fluorescence lifetime imaging *(Invited Paper)*

Ingo Gregor, Georg-August-Univ. Göttingen (Germany); Fred Wouters, Gertrude Bunt, Universitätsmedizin Göttingen (Germany); Jörg Enderlein, Georg-August-Univ. Göttingen (Germany)

The development of single-molecule fluorescence techniques has led to an enormous progress in quantitative cellular biophysics. However, the precise absolute quantification of concentrations, or even relative numbers, of several proteins at the same time within a single cell remains difficult. For many biomedical studies, this information can provide insight into intracellular correlations of protein levels. Such information is the key to understand e.g. cancer development and finding possible strategies for therapeutic intervention.

Using Fluorescence Lifetime Imaging Microscopy (FLIM) we are able to simultaneously quantify the relative levels of three important tumor-associated proteins within a single cell from immunofluorescence signals. Usually, the quantitative analysis of multicomponent FLIM data is time consuming and prone to errors if the lifetime decay patterns are complex (multi-exponential). Therefore, the development of a new approach to quantify FLIM data had central importance. Our method is fast and quantitatively precise even in the case of complex fluorescence decays. Based on simulated data-sets we determine error levels and compare the performance of the procedure to Phasor analysis and Maximum-Likelihood estimator.

The algorithm was easily extended to include spectrally resolved FLIM (sFLIM) data, offering the potential to quantify up to ten fluorescence markers simultaneously.

8950-15, Session 4

Metal-induced energy transfer: measuring quantum yields and molecular distances

Narain V. S. Karedla, Alexey I. Chizhik, Jörg Enderlein, Georg-August-Univ. Göttingen (Germany)

When placing a fluorescent molecule close to a metal, its fluorescence properties change dramatically. In particular, one observes an angular distribution of radiation which is completely different from the molecule in a homogeneous environment, a modification of its emission spectrum, and a strongly modified lifetime of its excited state (Purcell effect). This is due to the efficient electromagnetic coupling of the excited state to surface plasmons in the metal, which is similar to Förster Resonance Energy Transfer (FRET), where the energy of an excited donor molecule is transferred into the excited state of an acceptor molecule. We call this effect metal-induced energy transfer or MIET. Recently, we have used this effect to measure absolute values of quantum yield of fluorescing emitters. By placing a solution of the emitters into a metallic nano-cavity and monitoring the lifetime modification as a function of the cavity size, one can determine the radiative and non-radiative transition rates as well as estimate rotational diffusion coefficients. This method is even applicable for complex system showing multi-exponential decay behavior, where it allows for determining the quantum yield of emission for each sub-state separately, or for inhomogeneous mixtures of several emitters, where it allows for obtaining quantum yield values of each emitter species in the mixture. As far as we know, not other existing method is capable of doing that.

However, MIET can be used also for localizing fluorescent emitters with nanometer accuracy. The MIET-coupling between an excited emitter and a metal film is strongly dependent on the emitter's distance from the metal. We have used this effect to localize, with nanometer accuracy, tubulin molecules above a metallic surface. Here, we will present an extension of this method which we have used for mapping the basal membrane of live cells with an axial accuracy of ~3 nm. The method is easy to implement and does not require any change to a conventional fluorescence lifetime microscope; it can be applied to any biological system of interest, and is compatible with most other super-resolution microscopy techniques which enhance the lateral resolution of imaging.

8950-16, Session 4

The regulatory switch of F1-ATPase studied by single-molecule FRET in the ABELtrap *(Invited Paper)*

Samuel D. Bockenhauer, Stanford Univ. (United States); Thomas M. Duncan, SUNY Upstate Medical Univ. (United States); William Esco Moerner, Stanford Univ. (United States); Michael Börsch, Friedrich-Schiller-Univ. Jena (Germany)

F1-ATPase is the soluble portion of the membrane-embedded enzyme FoF1-ATP synthase which catalyzes the production of adenosine triphosphate in all types of cells. In reverse, the F1 part can also hydrolyze ATP quickly at three catalytic binding sites. Therefore, catalysis of 'non-productive' ATP hydrolysis by F1 (or FoF1) has to be prevented in the cell. The central epsilon subunit is thought to control and block ATP hydrolysis by mechanically sticking its C-terminus into the rotary motor of F1. We investigate this proposed mechanism by labeling F1 specifically with two fluorophores to monitor the C-terminus of the epsilon subunit by Förster resonance energy transfer. Single F1 molecules are trapped in solution by the Anti-Brownian electrokinetic trap which keeps the FRET-labeled F1 in place for extended observation times of several hundreds of milliseconds, limited by photobleaching. FRET changes in single F1 and FRET histograms for different biochemical conditions are compared to evaluate the proposed regulatory mechanism.

8950-17, Session 4

Molecular modeling of the Förster resonance energy transfer in genetically encoded fuse proteins

Maria G. Khrenova, Lomonosov Moscow State Univ. (Russian Federation) and A.N. Bach Institute of Biochemistry (Russian Federation); Alexander S. Goryashchenko, Victoria V. Zherdeva, A.N. Bach Institute of Biochemistry (Russian Federation); Alexander V. Nemukhin, Lomonosov Moscow State Univ. (Russian Federation); Alexander P. Savitsky, A.N. Bach Institute of Biochemistry (Russian Federation)

Fluorescence imaging is a powerful tool to investigate biochemical and biophysical processes in vitro and in vivo at the molecular level. Förster resonance energy transfer is one of the most sensing mechanisms to monitor the changes happening with target molecules. Herein we present the experimental and computational study of the genetically encoded FRET systems that are fuse proteins composed of two red fluorescent proteins joined with linkers that contains motif which can be distinguished by selected type of enzyme. By now we are able to predict possible bound structures of the fuse proteins and estimate their impact to all possible conformations. The understanding of preferred conformations can help improving the efficiency of the selected pair of donor and acceptor. Otherwise we can explain the reasons that prevent from enhancement of the FRET sensor based on the selected fluorescent proteins. We use high level computational methods, such as density functional theory for calculations on the ground state potential energy surface and multiconfigurational methods for the excited state potential energy surfaces. These calculations provide us with the important information on the transition dipole moments that are necessary to calculate FRET efficiency. Molecular docking procedure is used to calculate possible bound structures and molecular dynamics to predict possible unbound conformations. Furthermore, we found experimentally and explained through our theoretical model that the oligomerization state of acceptor influences the FRET efficiency. Observed fluorescent lifetimes in buffer solutions are shorter than that in cells that is explained by different refractive index in these media.

8950-18, Session 5

Accelerating localization microscopy

Patrick Fox-Roberts, Susan Cox, King's College London (United Kingdom)

Localization microscopy is a powerful tool for imaging structures at a lengthscale of tens of nm, but its utility for live cell imaging is limited by the time it takes to acquire the data needed for a super-resolution image. The acquisition time can be cut by more than an order of magnitude by using a fitting algorithm which can analyse dense data, effectively trading off acquisition and processing time. We have developed two methods which allow different tradeoffs to be made.

Modelling the entire localization microscopy dataset using a Hidden Markov Model allows localization information to be extracted from extremely dense datasets. This Bayesian analysis of blinking and bleaching (3B) is able to image dynamic processes in live cells at a timescale of a few seconds, though it is very computationally intensive, requiring at least several hours of analysis.

An alternative statistical modelling approach automatically determines the number, position, and brightness of fluorescing molecules within a particular image region. The background noise in each image is treated as a parameter of the model itself and optimised directly, removing the need for a hand-set threshold. Individual update stages of the algorithm can be run in parallel, which is one factor that allows this algorithm to run an order of magnitude faster than 3B.

We compare our methods to other high density algorithms, and discuss the artefacts which can occur during reconstruction of the super-resolution image. The methods are demonstrated on various live cell systems, including cardiac myocytes and podosomes.

8950-19, Session 5

Optical processing techniques for measuring the position and orientation of single molecules, with applications to superresolution imaging

Adam S. Backer, Mikael P. Backlund, Alex R. Diezmann, William Esco Moerner, Stanford Univ. (United States)

Single-molecule active control microscopy (SMACM) has shattered the optical diffraction limit, extending the resolving capabilities of fluorescence microscopy by an order of magnitude. However, SMACM imaging techniques hinge upon the accurate localization of single molecules from their fluorescence emission patterns. If our ability to 'super-localize' single molecule emitters is compromised, the quality of our images will inevitably suffer. Recently, a number of groups have warned that the standard practice of fitting Gaussian PSFs to a dataset of single-molecule images may introduce systematic localization errors—even though the precision of position measurements will improve as more photons are detected, the estimates will be irreparably biased, because the wrong theoretical model is being applied to the acquired data. The discrepancy arises from the fact that fluorescence from a single molecule is in general not isotropic! Instead, the emission pattern usually resembles that of a classical electric dipole. As a result, single-molecule images will vary as a function of a molecule's orientation with respect to the microscope objective, and the centroid of these images will appear to shift as a function of both orientation and the objective's defocus. We will present a Fourier-plane optical processing system developed by our lab to measure the orientation of single molecules, and if need be, correct the position errors that may result when performing superresolution imaging. Through phase modulation and the simultaneous acquisition of polarization data, our technique alters the standard microscope PSF into a form that permits orientational effects to be readily sensed and corrected.

8950-20, Session 5

mRNA quantification via second harmonic super resolution microscopy

Jing Liu, Il-Hoon Cho, Ulhas Kadam, Joseph Irudayaraj, Purdue Univ. (United States)

Increasing evidence suggests that phenotypic heterogeneity plays a critical role in the onset and progression of cancer; variation of the transcription of key genes in single cells occurs to trigger loss of tissue

homeostasis. Cell-specific information on quantity and localization of these transcripts in single-cell level are critical to the assessment of cancer risk, therapy efficacy, and effective prevention strategies. However, current available technologies, except the RNA fluorescence in situ hybridization (FISH) method, mostly rely on cell extraction that inherently destroys the tissue context and provide only average expression levels from cell populations or whole tissues. Although the RNA FISH method can quantify transcripts and provide their localizations in single cells, its drawbacks restrict quantifying single short transcripts beyond the diffraction limit. In this paper, we proposed a novel detection schematic, second harmonic generation (SHG) super-resolution microscopy (SHaSM), to detect single short mRNA transcript, Her2 mRNA, beyond the diffraction limit; and quantify them in single Hela, MCF7, and SKBR 3 cells. Nano-sized SHG crystals, barium titanium oxide BaTiO3 (BTO), were functionalized with two complimentary strands of Her2 mRNA after the chemical surface-modification. Dimer schematics was used to improve the specificity of detection and quantification, where two BTO monomers bind to the Her2 mRNA to form a dimer and observed via the SHaSM. SHaSM is able to detect single BTO nanocrystal with ~20 nm spatial resolution, and differentiate BTO dimers (Her2 mRNA) from BTO monomers (non-specific bounded BTO nanocrystal) with high specificity. SHaSM indicates that the average expression level of Her2 mRNA in single Hela, MCF7, and SKBR 3 cells is 0, 5, and 600 per cell, respectively, which agrees with the theoretical prediction.

8950-21, Session 5

Full-field nonlinear structured illumination microscopy with STED

Han Zhang, Ming Zhao, Yu Li, Leilei L. Peng, College of Optical Sciences, The Univ. of Arizona (United States)

Structured illumination microscopy (SIM) is a fast full-field imaging method. The linear SIM surpasses the lateral resolution limit by a factor of two. When combined with a nonlinear dependence of the fluorescence emission rate to a patterned illumination, SIM can improve the resolution further to <100 nm. Photoswitchable and excitation saturated SIM (SSIM) had been previously demonstrated. We report a new nonlinear SIM method that utilizes the stimulated emission depletion (STED) effect. Compare with the previous nonlinear SIM approaches, STED-SIM has the advantage of fast switching response, negligible stochastic noise in switching and unlimited resolution in theory.

In addition, previous nonlinear SIM methods require the 1D structured illumination pattern to be rotated at multiple angles, which slows down the image acquiring due to mechanical constrains. We utilize a 2D grating to generate a 2D structured pattern, which is shifted in high speed by piezo stages. The imaging speed of 2D STED-SIM imaging is therefor only limited by the camera speed.

The STED-SIM microscope was tested on fluorescent beads samples and achieved full field 2D imaging at the speed of 2s / frame with <60 nm resolution. Imaging experiments of biological samples are under way.

8950-23, Session 6

Time and polarization resolved CW STED photodeselection in molecular probes

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Time resolved fluorescence intensity and anisotropy measurements are combined to monitor the evolution of continuous wave stimulated emission depletion (CW STED) in fluorescent proteins and exogenous fluorophores in a confocal microscope.Single photon pulsed excitation at 495nm is coupled with variable power CW depletion using diode lasers and a tunable frequency doubled VECSEL system. The fluorescence dynamics are found to strongly depend on the order created by the initial photoselection process and to a lesser degree the extent of orientational relaxation during the excited state lifetime. The technique is valuable in optimising low power CW STED and associated super resolution imaging applications. It is also useful as a means of determining the relative dipole strengths of the emitting states involved in bi-exponential fluorescence decays allowing a clearer understanding of restrictive pathways in non-trivial FRET dynamics [1].

[1] Restricted State Selection in Fluorescent Protein Fo?rster Resonance Energy Transfer, T A. Masters, R. J. Marsh, D. A. Armoogum, N. Nicolaou, B. Larijani and A. J. Bain*, J. Am. Chem. Soc. 2013, 135, 7883?7890

8950-24, Session 6

Superresolution optical fluctuation imaging using unknown patterned illumination

MinKwan Kim, Chung Hyun Park, YongKeun Park, Yong-Hoon Cho, KAIST (Korea, Republic of)

Resolution in conventional light microscopy is fundamental limited by Abbe's law of the diffraction barrier. To distinguish two objects, they need to be apart from each other at least half the wavelength of light. To overcome this diffraction limit in optical imaging, several super-resolution methods have been developed. Among them, Super Resolution Optical Fluctuation Imaging (SOFI) method developed by T. Dertinger et al [1], probvides optical images beyond the diffraction limit, employing higher-order statistical analysis of independent temporal optical fluctuation induced by blinking phenomena from fluorophore. SOFI is a technically simple and versatile method for super-resolution imaging. However, because SOFI utilizes blinking property of fluorophore, there are practical challenges and limitations in employing SOFI for some application, including the limitation of available fluorophores for SOFI, the requirement for a high-speed camera, and low signal-to-noise ratio, mainly caused by the difficulty in controlling the blinking of fluorophores. To solve these limitations, we proposed a new approach combining SOFI with randome patterned illuminations to create illumination-induced optical fluctuation to increased resolution. The induced fluctuation is used instead of blinking fluctuation because the induced fluctuation is possible to control unlike blinking of fluorophore. Furthermore, This technique allows enhancement of signal to noise ratio by changing the illumination pattern, giving us a chance to achieve the high signal-to-noise SOFI using induced fluctuation with various fluorophores.

8950-25, Session 6

3D STED nanoscopy with adaptive optics

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Stimulated emission depletion (STED) microscopy is an increasingly widely used tool for super resolved (SR) imaging inside biological samples. To date, however, most STED microscopy has been carried out realising super-resolved imaging in a plane parallel to the coverslip. It is often desirable to image in alternative planes, e.g. when visualising the immune synapse (IS) between a natural killer cell and its target cell, for which the synapse is typically aligned in a plane perpendicular to the coverslip. We present here a novel 3-D STED microscope to realise super-resolved imaging in any plane. It utilizes a spatial light modulator (SLM) that provides inherently collinear and complementary depletion beam profiles to realise 3-D STED and also enables the correction of

aberrations induced by both instrument and sample. This instrument also incorporates fluorescence lifetime imaging for multilabel SR microscopy. We have applied 3-D STED to demonstrate the first super resolved images of the IS between two interacting cells in their natural state and have also applied it to 3-D imaging of fluorescent nitrogen vacancy (NV) defects in diamond. 3-D super-resolved imaging is challenging because of sample induced optical aberrations that increase with depth but we are able to program our SLM to precompensate for spherical aberrations in index mismatched samples and have applied this to SR imaging in bulk diamond and through up to 70 microns of glycerol.

8950-53, Session 6

STED microscopy robust and simple *(Invited Paper)*

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Newly available opto-electronic and opto-mechanical elements and design principles enable compact and robust, virtually alignment free optical setups for multi color STED microscopes.

The QuadScanner design does not require any pupil in the optical beam path. Neither complex scan lenses nor extra pupil planes therefore are required in the beam path. This leads to an extremely short and robust beam path. Also the easySTED wave plate does not require a pupil in the beam path and makes the alignment of the excitation with respect to the STED beam obsolete. New pulsed triggerable STED lasers allow the combination with white light fiber lasers as excitation sources. An essentially color independent directional beam splitter allows the usage of white light lasers for excitation without the need for special dicroics. System control via a single LABVIEW programmed FPGA board makes multi color imaging, gating, lifetime imaging and laser synchronization easy and flexibly adaptable.

The new STED microscope design strategies will be explained and multi color STED image results will be shown.

8950-54, Session 6

STED microscopy of neuronal tissue

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No Abstract Available

8950-26, Session 7

Design and development of BODIPY-based photoswitchable fluorophores to visualize cell signaling with multispectral super resolution microscopy

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Super resolution microscopy (SRM) has overcome the historic spatial resolution limit of light microscopy, enabling fluorescence visualization of cellular structures and multi-protein complexes at the nanometer scale. Using single-molecule localization microscopy, the precise location of a stochastically activated population of photoswitchable fluorophores is determined during the collection of many images to form a single image with resolution of ~10-20 nm, an order of magnitude improvement over conventional microscopy. However, single-molecule SRM is currently limited to a maximum of 4-color emission-based imaging in a single sample due to suboptimal photoswitchable fluorescent probes and conventional bandpass filtering. In the current work a library of novel BODIPY-based fluorophores was synthesized using a solid phase synthetic platform to create a set of photoswitchable fluorophores that can be excited by 4-6 laser lines but emit throughout the spectral range (450-850nm) enabling multispectral super resolution microscopy (MSSRM). The photoswitching properties of all new fluorophores were quantified for the following key photoswitching characteristics: (1) the number of photons per on cycle, (2) the percentage of time the fluorophore spends in the on and off states, and (3) the susceptibility of the fluorophore to photobleaching. Utilizing the optimal novel BODIPY fluorophores, the HER2 cell signaling pathway has been imaged in HER2 overexpressing breast cancer cell lines that are both resistant and responsive to HER2 targeted therapy. As demonstrated through HER2 cell signaling imaging, MSSRM with optimized photoswitchable fluorophores will enable quantification of protein-protein interactions at the nanometer scale and revolutionize nanometer scale imaging technology for the scientific community.

8950-27, Session 7

A novel aligning method for stimulated emission depletion microscopy using fluorescence lifetime distribution

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Stimulated emission depletion (STED) microscopy provides diffraction-unlimited resolution while inheriting good properties of non-invasion, high sensitivity and high specificity from confocal fluorescence microscopy. It has now become one of the most promising techniques for biological imaging. However, STED microscopes are not widely available due to the complexity instrumentation and the strict alignment at nanoscale.

Optimal resolution by STED microcopy requires precise alignment of the doughnut-shaped depletion focus to the excitation focus. Conventionally, the alignment has been achieved by imaging scattered laser light from gold nanobeads and adjusting two focuses until the two PSFs align. However, a scattering imaging module needs to be added to this fluorescence system, which makes the system more complex and costly. Also, PSF of each beam should be imaged separately, which may involve drift errors either from the sample or the system. An aligning method using fluorescent nanobeads has been proposed to get rid of the scattering imaging module using fluorescence intensity distribution, but it still suffers from drift errors caused by separate imaging. Also, alignment precision based on fluorescence intensity distribution requires a proper radio of the excitation power and the depletion power. Theoretically, fluorescence lifetime distribution can be used in STED's alignment. A doughnut-shaped depletion focus will lead to the resulted fluorescence spot with longer lifetimes in the center and shorter ones in the edge, which has little to do with the excitation intensity distribution. In this paper, we propose a novel aligning method using fluorescence lifetime distribution. A lifetime distributed image of fluorescent nanobeads can be obtained by a CW-STED system with a TCSPC module. By adjusting the center of the long lifetime PSF to the center of the fluorescence spot, relative spatial alignment of the depletion and the excitation foci in all three dimensions can be achieved by a single frame without introducing drifting errors caused by separate imaging. No mismatch between scattering and fluorescence imaging modes is introduced by avoiding the scattering imaging module. By this method, a resolution of 38 nm with time-gated detection has been achieved. It also has the potential to be used in a multi-color CW-STED system and to be automated.

8950-28, Session 7

3D-superlocalization microscopy of single FoF1-ATP synthase in living Eschericia coli

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FoF1-ATP synthases are membrane-embedded protein machines that catalyze the synthesis of adenosinetriphosphate. The enzymes are located in the inner mitochondrial membrane, the thylakoid membrane of chloroplasts or the plasma membranes of bacteria. The mitochondrial FoF1-ATP synthases are arranged as dimers or oligomers, respectively, and are specifically distributed as shown by electron microscopy. Here, we explore the spatial distribution and measure diffusion properties of bacterial FoF1-ATP synthases in living E. coli cells under physiological conditions. Using 3D-STORM (STochastic Optical Reconstruction Microscopy) and PALM (PhotoActivated Localization Microscopy) in epi- and TIR-illumination as well as SIM (Structured Illumination Microscopy), we evaluate the optimal fluorescence label for our single-molecule FRET studies of catalytic subunit rotation in FoF1-ATP synthase in vivo.

8950-29, Session 7

Photocontrollable rhodamine spirolactams for single-molecule imaging

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There is a persistent need for bright small-molecule fluorophores with photocontrollable emission for applications like single-molecule based super-resolution techniques. These methods extend the resolution of the optical microscope by roughly an order of magnitude. The resolution enhancement scales as $1/\sqrt{N}$ (where N is the number of detected photons), and small-molecule fluorophores emit an average of ten-fold more photons than the ubiquitously used fluorescent proteins. Additionally, the photophysical properties of small-molecule fluorophores can be readily modified using organic synthesis. We have synthesized and characterized a set of photoswitchable rhodamine spirolactam derivatives with the aim of optimizing these molecules for super-resolution microscopy. Rhodamine spirolactams belong to a class of photoswitchers with two distinct isomers. The non-fluorescent, “closed” isomer converts to the fluorescent “open” isomer upon the absorption of UV light. The open isomer will thermally revert to the more stable closed isomer or irreversibly photobleach. Our optimization effort focused on (1) shifting the photoactivation process to wavelengths greater than 400 nm, in order to avoid the use of UV activation sources which can damage cells, and (2) increasing the water-solubility of the fluorophores for increased biocompatibility. Both bulk and single-molecule measurements quantified the photophysical differences between varying structures. Increasing the conjugation in the spirolactam portion of the molecule shifted the absorption of the closed form into the visible range. Adding charged substitutents increased water-solubility. Using a tagging moiety, the optimized rhodamine lactam was imaged in a biological system with three-dimensional super-resolution.

8950-30, Session 8

Superresolution imaging of ciliary microdomains in isolated olfactory sensory neurons using a custom STED microscope *(Invited Paper)*

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We performed super-resolution imaging of isolated olfactory sensory neurons (OSNs) using a custom-built Stimulated Emission Depletion (STED) microscope. The design for the STED microscope is based on the system developed in the laboratory of Dr. Stefan Hell (Johanna Bückers, et. al., Opt. Express 19, 3130-3143 (2011)). Our system is capable of imaging with sub-diffraction limited resolution simultaneously in two color channels (at Atto 590/Atto 647N wavelengths). A single, pulsed laser source (ALP; Fianium, Inc.) generates all four laser beams, two excitation and two STED. The two STED beams are coupled into one polarization maintaining (PM) fiber and the two excitation beams into another. They are then collimated and both STED beams pass through a vortex phase plate (RPC Photonics) to allow shaping into a donut at the focus of the objective lens. The beams are then combined and sent into an inverted research microscope (IX-71; Olympus Inc.) allowing widefield epifluorescence, brightfield and DIC imaging on the same field of view as STED imaging. A fast piezo stage scans the sample during STED and confocal imaging. The fluorescent signals from the two color channels are detected with two avalanche photodiodes (APD) after appropriate spectral filtering. We performed STED imaging on immunolabelled isolated OSNs tagged at the CNGA2 and ANO2 proteins. The STED microscope allows us to resolve ciliary CNGA2 microdomains of ~54 nm that were blurred in confocal.

8950-31, Session 8

Image scanning microscopy (ISM)

Jörg Enderlein, Georg-August-Univ. Göttingen (Germany)

Recent years have seen an explosion in the development of new enhanced and super-resolution microscopy techniques. One of them is Structured Illumination Microscopy (SIM) which combines patterned illumination with wide-field detection for improving the image resolution by two-fold. The advantage of SIM is that it works on any sample without the need of special labels or fixation. The drawback of SIM is its technical complexity and correspondingly high cost, and its sensitivity to optical and sample imperfections, which easily introduce artifacts into a final image. Some years ago, we have experimentally demonstrated that one can achieved the same resolution enhancement by equipping a conventional laser scanning confocal microscope (LCSM) with an imaging detector (C. B. Müller and J. Enderlein, Phys. Rev. Lett. 104, 2010, 198101). At each scan position of the LCSM, one records a small image of the illuminated region, and by applying a simple algorithm to all acquired images, one then computes a final image with doubled resolution. We have called this technique Image Scanning Microscopy or ISM. Now, we have adapted the original idea if ISM to a Confocal Spinning Disk (CSD) microscope, which has significantly increased imaging speed, allowing for a final ISM image rate of 1 Hz. We discuss the technical details of the CSD-ISM and present numerous biological applications with three-dimensional enhanced-resolution and high-contrast multicolor images of cells and tissues.

8950-32, Session 8

Development of fluorescence probes for super-resolution imaging based on intramolecular spirocyclization

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Super-resolution imaging techniques to break the diffraction limit have developed rapidly in recent years. Among them, single-molecule localization microscopy (SLM) including (d)STORM, (f)PALM, and GSDIM enables us to construct super-resolution images by repeating detection and high precision localization of individual fluorophores. Here, we report fluorescence probes suitable for SLM based on thermal intramolecular spirocyclization. Rhodamine derivatives bearing an intramolecular nucleophile exist in thermal equilibrium between fluorescent open form and non-fluorescent spirocyclic form in the ground state, which we focused on to utilize for spontaneous fluorescence blinking for SLM. For this purpose, we needed a fluorophore with which only a small subset of them should exist in fluorescent state, lasting for at least

several frames to detect enough photons. Therefore, we designed, synthesized and evaluated a series of rhodamine derivatives bearing various intramolecular nucleophiles and/or fluorophores, and found that chemical structures significantly influenced on an equilibrium constant of intramolecular spirocyclization (pKcycl) and a rate constant of ring-closure reaction (k). We confirmed that one of developed dyes spontaneously and repeatedly blinked with proper average fluorescence life time of fluorescent state. Furthermore, microtubules labeled with the dye could be reconstructed with sufficient spatial resolution without any additives.

8950-52, Session 8

Using dSTORM to probe the molecular architecture of filopodia

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IRSp53 is a Cdc42 effector and a member of the Inverse-Bin-Amphiphysins-Rvs (I-BAR) domain family which can induce negative membrane curvature. IRSp53 generates filopodia by coupling membrane protrusion (I-BAR domain) with actin dynamics through its SH3 domain binding partners. We find that Dynamin 1 (Dyn1), a large GTPase associated with endocytosis, is a novel interacting partner of IRSp53 that localises to filopodia. . Using rapid time-lapse TIRF microscopy we show that Dyn1 localized to a subcellular region just behind Mena at the leading edge, or in filopodial tip complexes when co-expressed with IRSp53. Dyn1 knockdown showed a reduction in filopodia formation and addition of dynasore, a non-competitive inhibitor of Dynamin GTPase, induced a loss of filopodial dynamics. Dyn1-GFP was strongly localized in the filopodial shaft during the early phase of elongation, after which it moved rearward, suggestive of a role in early filopodia assembly. Mena and Eps8, accumulate at the tip complex in sequence and are involved in filopodial extension and retraction, respectively. Using dSTORM we investigate the molecular architecture of filopodia and show how protein distribution links to function. Taken together, this data allows us to put forward a model for filopodia.

8950-34, Session PSun

Doubling the lateral resolution of confocal scanning laser microscope using virtually structured detection

Rongwen Lu, Benquan Wang, Qiu-Xiang Zhang, Xincheng Yao, The Univ. of Alabama at Birmingham (United States)

Structured illumination has been substantially used to improve the lateral resolution beyond the diffraction limit by a factor of two in the wide field fluorescent microscopy. However, practical application of the wide field structured illumination in live thick tissue is challenging. The illumination pattern could be deteriorated by the out-of-focus background. In addition, reconstructed images suffer from possible phase errors in sequential illumination patterns.

The purpose of this study was to develop a phase-artifact free virtually structured detection (VSD) method for confocal scanning laser microscope (SLM) to achieve super-resolution in live thick tissue, which could be used in either intrinsic (e.g., reflectance or transmission) or fluorescence imaging. Instead of using a single element detector and a confocal pinhole on front of it in conventional SLM, a spatially resolved detector was employed to record two-dimensional diffraction maps. Confocal configuration was achieved using virtually synthesized pinholes. Then those individual maps were modulated by digital sinusoidal masks before spatial integration to shift beyond-diffraction-limit band into the pass-band. The super-resolution images were recovered using the same algorithm as in the wild field structured illumination microscope. The sub-diffractional resolution of the VSD based confocal SLM was validated by 1) computer simulation; 2) experiments on the standard optical test target; and 3) experiments on the freshly isolated frog retina. We anticipate further development of VSD promises an easy, low-cost and phase-artifact free strategy for in vivo super-resolution imaging.

8950-35, Session PSun

SERS fiber probe fabricated by femtosecond laser with lateral surface silver coating on micro-fiber tips

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We report a method to fabricate surface enhanced Raman scattering (SERS) fiber probe by femtosecond laser micromachining on a microfiber tip. Multimode optical fibers with a 105/125 μm core/cladding diameter are tapered by sweeping flame method to result in microfiber tips with outer diameter of 6 to 15 μm. Femtosecond laser pulses with 0.8W output power and 250K repetition rate is used to form nanostructures on the cleaved endface of the microfiber tip. The laser ablation resulted endface is then activated by silver sputter coating. Microfiber with core diameter less than 10 μm cannot confine optical energy efficiently inside the fiber. To confine the excitation and reflection signal within the tapered core of the microfiber tip, the probe is side-coated with silver plating. A scanned mode field distribution measurement shows that the plating can contribute to confine the light emission outside the probe end. High quality SERS signal of Rhodamine 6G molecules with various concentrations is detected through back excitation as well as back collection from a lead in fiber of up to several meters long. The small size of the SERS micro-probe is promising for intra-cellular or even single cell detection, while the back excitation and collection setup make it suitable for remote sensing applications.

8950-36, Session PSun

Imaging antimicrobial peptides acting on nano structured lipid bilayer membranes using multimodal optical microscope

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We study antimicrobial peptides (AMPs) disrupting supported lipid bilayer membranes using a multi-modal optical microscope that has a fluorescence confocal microscope and a fluorescence lifetime imaging microscope(FLIM). To mimic bacteria cell membranes, supported lipid bilayer membranes with different ratios of charged lipids are prepared on the glass surface using a Langmuir-Blodgett technique. A confocal fluorescence microscope is used to visualize structural information of nano-structured lipid bilayer membranes with high resolution, and simultaneously, FLIM is used to image biochemical environment near the membrane. The multi-modal optical microscope with two modes detect nano-structured lipid bilayer membranes without moving a sample stage. We study the change of AMPs' activity on the model cell membranes by changing pH, salt concentrations, and lipid composition of the membranes. The lifetime of a fluorescence probe detects such changes, therefore it is possible to quantify the interactions between lipid bilayer membranes and AMP under different environmental conditions. We combine information obtained by two imaging techniques to correlate the activity of AMPs and environmental conditions. The influence of different environments on the activity of AMPs for nano-structured lipid bilayer membranes are modeled .

8950-37, Session PSun

Intrinsic blinking of red fluorescent proteins for superresolution imaging

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Superresolution microscopy proved to be a very exciting technology in the last decade. It has allowed to overcome the diffraction limit and look into the ultrastructure of living cell. Such techniques as stochastic optical reconstruction microscopy/photoactivated localization microscopy (STORM/PALM) have been able to retain the advantages of fluorescence microscopy while approaching the resolving power of electronic microscopy. Recently more photoactivatable fluorescent proteins have been reported as tools for single-molecule resolution imaging. Particularly, intrinsic blinking of certain green and yellow fluorescent proteins has been exploited to achieve superresolution. We tested various monomeric orange and red fluorescent proteins for stochastic switching from a dark state to a bright fluorescent state. Transfected NIH 3T3 and HeLa cells expressing fluorescent proteins fused with cytoskeletal proteins were studied. Image acquisition was carried out on the Nikon N-STORM system. We showed that relatively low power (10 percent) of 561 nm laser was sufficient to initiate blinking of certain fluorescent proteins. Selected proteins were further examined for the quantity of localized molecules after the image reconstruction. It was established that we could localize at least 150 blinking events per frame and achieve minute-scale superresolution of living cells.

In summary, we for the first time described intrinsic blinking of bright red fluorescent proteins sufficient for STORM/PALM image reconstruction. Stochastic single-molecule resolution imaging of actinin, zyxin, profilin and other cytoskeletal proteins was performed. We also demonstrated minute-scale superresolution imaging of living cells with a weak laser irradiation.

8950-38, Session PSun

Breaking the diffraction limit of wide-field microscopy using frequency shift

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Structured illumination microscopy (SIM) stands out for its super-resolution and three dimensional (3D) reconstruction capability. Although SIM is initially proposed to image fluorescent samples, its applications can potentially be extended to the mark-free area. However, compared with other super-resolution approaches, structured illumination can only double the resolution of a microscope, because the illumination pattern is still diffraction limited. This problem can partially be solved by introducing saturation effect, but anyone who uses this method also has to face the risk of irreversible damage to the sample.

Following the similar fundamental of SIM, we propose a new super-resolution method that is based on frequency shift principle and evanescent field illumination. We theoretically and experimentally confirmed that, when the sample is illuminated by the evanescent field with a determined direction, the spatial frequencies of the image can be shifted along the illumination direction. Therefore, it is possible to obtain higher frequency components without expanding original passband of the system. Since the wave number of the evanescent field is not restricted by the diffraction limit of the system, our method can supply a larger step length for frequency shift, which corresponds to better resolution. To expand the spatial frequency scale in 2D, a series of images of the sample can be recorded under different illumination directions, and the spatial frequencies of all frames are combined. The final image with sub-diffraction-limited details will originate from the inverse-Fourier transform of the combined frequencies. Compared with other super-resolution methods, this new idea possesses promising merits. It provides a simpler configuration and a wider viewing field, and does not rely on labelling, thereby implying abundant application potentials, especially for the mark-free biological samples.

8950-39, Session PSun

Sub-diffraction-limited imaging by photobleaching imprinting microscopy (PIM)

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Fluorescence microscopy has been extensively used to gain a deeper understanding of cell and tissue biology. Despite widespread applications, fluorescence microscopy faces a fundamental bottleneck in its resolving capability — the optical diffraction limit. Due to this limitation, the achievable spatial resolution of conventional fluorescence microscopy is ~ 250 nm in the lateral direction, and ~ 500 nm in the axial direction. Since many cellular organelles, such as microtubules, actin fibers, and ribosomes, are smaller than this size, breaking the optical diffraction limit has been the holy grail of light microscopy over the past several decades.

To address this challenge, a wide array of methodologies has been introduced, allowing the fine structures of a biological cell or tissue to be revealed at the super-resolution level (Science, 316, p1153). However, most super-resolution techniques rely on specific fluorescent probes or require complicated optical illumination modules, limiting access by the general research community.

To provide a generic method that can be readily implemented on a standard light microscope with conventional fluorescent dyes, here we present photobleaching imprinting microscopy (PIM) for super-resolution fluorescence imaging. PIM works by first imprinting a pattern onto the sample through photobleaching, followed by light interrogation with another focused Gaussian laser beam. Using PIM, we demonstrated a lateral resolution of ~ 110 nm, more than a two-fold improvement over the optical diffraction limit. Additionally, we showed that PIM can reduce the out-of-focus light in tissue fluorescence imaging, considerably improving the image contrast.

8950-40, Session PSun

Subwavelength light manipulation via wavefront shaping in complex media

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The resolution limit using visible light is a limiting factor in many fields of research. This is especially undesirable for bio- and nano-photonics where imaging and focusing at the sub-diffraction limited regime is critical. Due to these limitations, various novel schemes have been developed allowing sub-diffraction limited imaging using visible light. However, fluorescent nanoscopic methods such as Stochastic Optical

Reconstruction Microscopy (STORM), PhotoActivated Localization Microscopy (PALM), or structured illumination rely on numerical post-processing to achieve super resolution and cannot excite or perturb sub-diffraction limited spots physically. Other methods such as Stimulated Emission Depletion (STED) microscopy emits fluorescence in sub-diffraction limited volumes but works only on systems where another beam can be used to de-excite the fluorescence.

In this work, we demonstrate that we can use multiple scattering to obtain a sub-diffraction limited focus at an arbitrary position. Due to the random structure of the highly scattering media, there are no restrictions on the physical position of the focus giving the system a high degree of freedom. Through this demonstration we propose that multiple scattering in biological tissue, which had been assumed to be unfavorable for imaging or light treatment, can on the contrary be used to steer and focus sub-diffraction limited light spots with arbitrary polarization or wavelength within its inhomogeneous structure. The wavelength of light is also shown to be independent on the size of the obtained focus which gives this method a broad range of spectral applicability as resonance is not its working mechanism.

8950-41, Session PSun

Reverse saturable scattering of a single gold nanoparticle

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Last year, we reported saturable scattering (SS) in an isolated plasmonic nanoparticle, providing a novel contrast agent without bleaching for superresolution microscopy. It has been extensively reported that absorption in plasmonic materials exhibits not only saturation, but also reverse saturation behaviors. The saturation behavior is due to the depletion of ground state, while the reverse saturation origins from excited state absorption or two-photon absorption. We have found that the intensity required for SS is similar to the intensity required for saturable absorption (SA). Plus the fact that scattering and absorption correlate with the real and imaginary parts of dielectric constant, we believe that SS shares the same physical origin with SA. In case it is true, we then expect to see, for the first time, reverse saturable scattering (RSS) of an isolated plasmonic particle by increasing excitation intensity.

In our experiment, a 561-nm laser, which is on the resonance of surface plasmon in 80-nm gold nanoparticle, is used in conjunction with a confocal microscope. When incident intensity increases from 10^4 W/cm^2 to 10^6 W/cm^2, the scattering intensity dependence evolves from linear, to saturation, and to reverse saturation sequentially. The intensity dependence in RSS region is significantly steeper than that in the linear region, and thus the full width half maximum of single-particle point spread function becomes less than 80 nm, which is beyond the diffraction limit. Our finding not only provides a possible explanation for SS in plasmonic particles, but also shows great potential for superresolution imaging applications.

8950-42, Session PSun

FPGA-based real-time multichannel correlator for high-throughput fluorescence correlation spectroscopy

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Fluorescence correlation spectroscopy (FCS) is a well-established technique to study binding interactions or the diffusion of fluorescently labeled biomolecules in-vitro and in-vivo. To monitor the signal fluctuations based on changes in the number of molecules within a detection volume of the order of fL, FCS has to operate at low concentrations, resulting in long acquisition time. In practice, FCS is performed with typical acquisition duration on the order of a few seconds to several minutes. However, faster acquisitions of FCS data are desirable in two cases: in high-content screening approaches, many molecules on reaction at different locations require simultaneous interrogation; also when observing fast evolving dynamic systems, diffusion parameters change as a function of time. Parallel FCS acquisition was thus developed, with the help of multi-pixel detectors and multi-spot excitation generation technique, which maps each excitation spot onto every target pixel of the detector. Simultaneous data acquisition and processing from the multi-pixel detectors is needed, demanding for a multi-input high efficiency correlator. To address this request we first developed a single channel FPGA-based correlator architecture featuring a lag-time ranging from 10ns up to 150ms. Because of its flexible and compact structure, the correlator can not only be extended to longer lag times but also expanded into a multichannel one, based on which simultaneous calculation of 32 multiple-tau autocorrelation functions is achievable. A 32-channel correlator is then designed and contained in a 32x1 SPAD array module, providing a compact and flexible instrument for advanced FCS experiments.

8950-43, Session PSun

Localization of single biological molecules out of the focal plane

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Since the behaviour of proteins and biological molecules is tightly related to the cell's environment, more and more microscopy techniques are moving from in vitro to in living cells experiments. Looking at both diffusion and active transportation processes inside a cell requires three-dimensional localization over a few microns range, high SNR images and high temporal resolution (ms order of magnitude). We developed an apparatus that combines different microscopy techniques to satisfy all the technical requirements for 3D tracking of single fluorescent molecules inside living cells with nanometer accuracy. To account for the optical sectioning of thick samples we built up a HILO (Highly Inclined and Laminated Optical sheet) microscopy system through which we can excite the sample in a widefield (WF) configuration by a thin sheet of light that can follow the molecule up and down along the z axis spanning the entire thickness of the cell with a SNR much higher than traditional WF microscopy. Since protein dynamics inside a cell involve all three dimensions, we included a method to measure the x, y, and z coordinates with nanometer accuracy, exploiting the properties of the point-spread-function of out-of-focus quantum dots bound to the protein of interest. Finally, a feedback system stabilizes the microscope from thermal drifts, assuring accurate localization during the entire duration of the experiment.

8950-44, Session PSun

Motionless focus control in PALM/STORM using adaptive optics

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Adaptive optics has recently been successfully used in PALM/STORM for 3D imaging of biological samples [1, 2]. In this case the 3D imaging capability is achieved by introducing a controllable amount of pure astigmatism using an adaptive optics device: a deformable mirror. Here, we present a new functionality of the deformable mirror in PALM/STORM. The full control of the focus of the microscope can be achieved using the deformable mirror so that both objective and the microscope stage are stationary. The step of the movement in axial direction can be as small as 10nm, which allows the creation of the calibration curves of the z position with high precision. PALM/STORM imaging deeper in biological samples is complicated by the presence of the spherical aberrations introduced by the mismatch of the refractive index, which greatly degrades the PSF and thus impedes the astigmatic approach for 3D imaging. Using iterative algorithms [3] those aberrations can be eliminated with the help of adaptive optics and the point spread function can be restored. We will present 3D PALM/STORM imaging of the cell deeper in the sample where the axial movement is entirely controlled by the deformable mirror.

1. Izeddin et al. (2012) Optics Express, 20, 4957-4967.

2. Specht et al. (2013) Neuron, in press.

3. Débarre et al. (2009) Opt. Lett. 34, 2495–2497

8950-45, Session PSun

Sample drift correction for STORM microscopy without use of fixed fiduciary markers

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Localization microscopy techniques such as STochastic Optical Reconstruction Microscopy (STORM) allow to obtain ten times improvement in image resolution compared with diffraction limit. However it requires several minutes of acquisition time and thus very sensitive to sample drift. Commonly used approach of drift compensation requires to introduce fixed fiduciary markers into the sample, which makes its preparation more cumbersome. Same time direct use of frames correlation may lead to poor results due to the fact that different subsets of fluorophores are imaged in different frames. We propose an algorithm able to compensate objects drift with the use of raw STORM data without introducing any additional markers into the sample. In case of sufficient number (>100) of events registered in every frame the algorithm allows to estimate sample drift between consecutive frames despite the fact that different subsets of fluorophores are imaged. Because no additional suppositions on drift time dependence were made, algorithm allows to compensate drift even in case of steep changes in its time dependence. In case of low number (<100) of events in each frame several consecutive frames may be concatenated to keep the algorithm performance.

8950-46, Session PSun

3D single-molecule tracking using one- and two-photon excitation microscopy

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Three dimensional single-molecule tracking (SMT) has revolutionized the way that we study fundamental cellular processes. By analyzing the spatial trajectories of individual molecules (a receptor or a drug particle), one can discern the internalization or transport dynamics of these molecules, study the heterogeneity of subcellular structures, and elucidate the complex spatiotemporal regulation mechanisms. Sub-diffraction localization precision, sub-millisecond temporal resolution and tens-of-seconds observation period are the benchmark of recent SMT techniques. Among the various approaches, confocal-feedback tracking is particularly advantageous as it enables a large axial tracking range, a high signal-to-noise ratios in detection, and time-resolved spectroscopy. We are currently building two molecular tracking systems in our labs. The first one is a duplicate of the Werner's tracking system developed at Los Alamos National Laboratory, which we denote as the 1P-1E-4D system (one-photon excitation scheme, one excitation beam, and four fiber-coupled detectors). The second one is a new design developed at University of Texas that uses two-photon excitation scheme and four demultiplexed excitation beams to spatiotemporally encode the position of a molecule. As only one single-photon detector is needed, we denote this new system as the 2P-4E-1D design (two-photon excitation scheme, four excitation beams, and one detector). Here we compare these two tracking systems using a Monte Carlo simulation based on the diffusion of a fluorescent molecule. Through our simulation, we have characterized the limitation of individual systems and optimized the system parameters such as magnification, z-plane separation, and feedback gains.

8950-47, Session PSun

Improved localization accuracy for fluorescence localization microscopy with statistical hypothesis testing of initial data

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In localization fluorescence microscopy techniques such as STochastical Optical Reconstruction Microscopy (STORM) image is formed as superposition of localized fluorophores. To localize each molecule one should define Region Of Interest (ROI) comprising an image of single molecule. This ROI is modeled as Point Spread Function (PSF) of a microscope plus uniform background. Position of a fluorescence molecule is calculated upon this model as a solution of an optimization problem. We applied chi-squared test to analyze how good such a model describes obtained ROI and found that only about 10% of events are described well for a significance level of 10%. Thus for the majority of events localization precision doesn't reach theoretically predicted values, however, it doesn't affected much by this discrepancy of model and experiment. Same time, localization positions, defined from ROI with lower number of chi-squared value shows lower dispersion. Thus this criterion can be used to improve resultant resolution of STORM images. We propose an algorithm based on these "trustworthy" events which allows to improve STORM images. The performance of the method was validated with several experimental images.

8950-48, Session PSun

Quantifying local density of optical states of nanorods by fluorescence lifetime imaging

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Interaction between emitters and environment, which focuses on the modulation of spontaneous emission and understandings of their optical as well as the electronic scattering and propagation characteristics, is the heart of nano-optics and photonics. The spontaneous emission as elucidated by Purcell, is inherently determined by the dipole transition momentum of the emitters, and externally influenced by the surrounding local density of optical states (LDOS). Quantification of LDOS becomes an important parameter for the characteristic of photonic structures. Previous methods to detect LDOS of a photonic structure required

complex nano-positioning in the near field. In this letter, we propose a facile platform to map the LDOS of single nanostructures without the intricacies involved in nanopositioning in the near field. Cadmium Telluride (CdTe) QDs as spontaneous emitters were conjugated to the surface of gold nanorods (AuNRs) by chemical modification. The distance between QDs and AuNRs could be controlled by a thin layer of silica at nanometer precision. The LDOS of the AuNR is determined by recording the spontaneous decay of the QDs. Our analysis indicates that the radiative decay of QD at the two ends of the nanorod is more enhanced (1.17 times greater) than that at the waist making it distinctly different, while the nonradiative decay was uniformly enhanced over the nanorod. To the best of our knowledge, our effort constitutes the first to map the LDOS of a nanostructure in the far-field and to provide clarity on the interaction mechanism between emitters and the nanostructure. The method presented will lead to a fast and convenient means to detect and quantify the photonic density for use in the design of photonic structures.

8950-49, Session PSun

Three-dimensional single particle tracking on apical surface of live cells using prism-coupled light sheet microscopy

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Single particle tracking has provided valuable insights into a variety of dynamic processes in living cells. Currently, common tracking techniques are based on epi, Confocal or TIRF microscopy. These techniques have difficulties to track single molecules in three-dimension at high spatiotemporal resolution. We developed a novel single particle tracking technique based on prism-coupled light-sheet microscopy (PCLSM). This tracking technique has combined benefits of superior signal to noise ratio (SNR), arbitrary penetration, large field of view and high spatiotemporal resolution. Z axis localization was achieved by introducing astigmatism into the emission optical path.

Using EGF receptor and A549 cells as a model system, we demonstrate tracking of single EGF molecules on the apical surface of live cell membranes. The whole “life time” of an EGF molecule, starting from its binding to EGF receptors until being internalized or photobleached, was recorded successfully. Fluorescently labeled EGF exhibits multiple diffusion behaviors on live cell membranes, which is represented by the broad distribution of EGF diffusion coefficient and dwelling time. At room temperature, the average diffusion coefficient of EGF on A549 cells was measured to be 0.13 μm2/s. To probe the effect of cholesterol on EGF transportation, we depleted cellular cholesterol with methyl-beta-cyclodextrin. Our results indicate that cholesterol depletion leads to a broader distribution of diffusion coefficients and an increase of the average diffusion coefficient at room temperature.

8950-50, Session PSun

Entropy-based calculations as a tool for denoising and resolution enhancement in fluorescence microscopy imaging

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Young Investigator Best Paper Competition BO403

We demonstrate a novel and relatively easy-to-use method to dramatically reduce noise and background contributions in advanced fluorescence microscopy experiments. The improved performance is achieved by pixel-wise calculation of the Shannon-Entropy value of a dynamic image sequence acquired with a standard widefield fluorescence microscope. This fluctuation-based analysis is inspired by thermodynamic considerations. The underlying idea is that the entropy value increases for systems with a large number of accessible energy states. In this case, intensity fluctuations originating from a range of photophysical or photochemical effects lead to an increased information content and thus increased entropy values. Calculating the pixel-wise entropy value results in an enhancement of the signal-to-noise ratio (SNR) of images with original SNRs of between 6-10 by a factor of 90-100. By comparing ECI (Entropy-based Contrast-enhanced Imaging) to stochastic reconstruction microscopy (STORM) and super-resolution optical fluctuation imaging (SOFI), we find that this method also bears substantial potential for enhancing fluctuation-based superresolution microscopies.

8950-51, Session PSun

Fluorescent nanodiamonds for ultrasensitive detection

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Fluorescent nanodiamonds (NDs) are new and emerging nanomaterials that have potential to be used as fluorescence imaging agents and also as a highly versatile platform for the controlled functionalization and delivery of a wide spectrum of therapeutic agents. Herein we are presenting potential applications of fluorescing NDs as novel ultrasensitive sensors for detecting macromolecular interactions. We utilize two experimental methods: TIRF, a relatively simple method based on the total reflection fluorescence and SPRF, fluorescence enhanced by resonance coupling with surface plasmons. We estimate that the SPRF method will be 100 times more sensitive than currently available similar detectors based on dyes. The ultimate goal of this research is to develop microarray platforms that could be used for sensitive, fast and inexpensive gene sequencing and protein detection.

Conference 8951: Optical Diagnostics and Sensing XIV: Toward Point-of-Care Diagnostics

Monday - Thursday 3 –6 February 2014

Part of Proceedings of SPIE Vol. 8951 Optical Diagnostics and Sensing XIV: Toward Point-of-Care Diagnostics

8951-23, Session PMon

Characterization of BxPC3-transplanted mice by hyperspectral autofluorescence imaging and Raman spectroscopy

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The developments of cell and molecular biology has been deepening and has proved particularly effective in the analysis of biological character of cancer. However, cancer has more complex structures than normal tissue so its form has not yet been fully clarified. Raman spectroscopy and autofluorescence imaging, are used to study the pancreatic cancer cell line (BxPC-3) - transplanted mice. The purpose of this study is to develop method to observe the characteristic of biochemical changing and histological type of subcutaneous tumor model in situ. Raman spectroscopy measurements were carried out at the different points in the tumor of living mouse under anesthetizing. As the results: Raman bands at 1658 and 1442cm-1 are assigned to amide I and CH2 bending modes of protein species, the scores of two principal components (PC1 and PC2) of the measured Raman spectra at some points, and the significant PC loadings were obtained. Autofluosescence imaging was adopted for clarification and imaging. It has potential as a method to assign the histological elements of the tissue. From the changing excitation wavelength and the observation wavelength, the fluorescence observed at 340 nm and 460 nm are assigned to NADH, at 400 nm and 620 nm are assigned to FAD or porphyrin. The differences among their spectra after comparing some points spectra of BxPC-3 as were analyzed and they show that their changing reflect the protein conformational alteration in the tumor tissue and associates with the calcifications.

8951-30, Session PMon

Imaging the information flow with single cell resolution in neuronal networks

Xiuli Liu, Shaoqun Zeng, Huazhong Univ. of Science and Technology (China)

Neural circuits is the physical basis for brain function, and monitoring the Information flow in neuronal networks is essential to reveal the circuit functions and find our how the brain works in normal or diseased state. To achieve this target, one might needs to develop novel technologies to detect every spike from every neuron and reveal the information flow. Currently techniques, such as functional magnetic resonance imaging and electrophysiological recording, have proved to be helpful in mapping brain activation for macro-circuits. However, these strategies are still hard to capture and resolve neuronal firing and then fail to trace the information flow in fine microcircuits due to their poor combined spatial-temporal resolution or lack of imaging. Optical diagnostics and sensing tools will play an important role in this topic as optical imaging methods might be the only candidate to monitor the brain activity with micron – level resolution i.e. single cell resolution. Recent progress in molecular biology has enabled translation the molecular event to an optical signal, and progress in information technology has enabled femtosecond pulse to image this signal inside the brain. Here we will show by combining the fast two-photon imaging technique, brain tissue calcium fluorescence labeling method, we are able to capture the calcium firing of neuronal networks, we then reconstruct the cellular activity with single spike precision, and image the information flow with single cell resolution in neuronal populations. This method will help to construct the functional connectomics of the microcircuits, and reveal the brain function.

8951-31, Session PMon

Novel noninvasive point-of-care device for real time hemoglobin monitoring

Ulrich Timm, Helge Gewiss, Jens Kraitl, Univ. Rostock (Germany); Kirstin Stupmann, German Red Cross Blood Donation Service (Germany); Michael Hinz, Sebastian Koball, Hartmut Ewald, Univ. Rostock (Germany)

During the perioperative period, which includes the period before surgery during surgery and after surgery (postoperative), it is essential to measure diagnostic parameters such as: blood oxygen saturation; hemoglobin (Hb) concentration; and pulse rate. The Hb concentration in human blood is an important parameter to evaluate the physiological condition of an individual, as Hb is the oxygen carrying component of red blood cells. By determining the Hb concentration, it is possible, for example, to observe intraoperative or postoperative bleeding, and use this information as a trigger for autologous/ allogenic blood transfusions. In blood donation center it is also an essential parameter for the decision regarding the acceptance of the donor. The paper will describe a novel multi-wavelength photometric method to measure the Hb concentration non-invasively. Clinic trails in blood donation centers and during the dialysis are done to prove and demonstrate the performance of the sensor system. The results are compared to the gold standard, the BGA measurement, and other POC devices which are invasive and non-invasive.

8951-32, Session PMon

Photon transport model of NIR light propagation in human finger tissue, for a point-of-care blood pressure measuring device

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Leman Micro Devices is developing a medical device that directs Near Infra Red light towards the finger artery, and detects the scattered light by a photodiode. The light passes through different layers of the finger tissue and undergoes multiple scattering events, and some of it passes through the radial artery. A change in the detected signal during each heartbeat can be attributed to the changing volume of the artery from systole to diastole. This data can be used to calculate the systolic and diastolic blood pressure.

Our paper first presents a theoretical model of the optical properties of the finger tissue and establishes the device geometries. Taking these properties into account we perform a stochastic simulation of NIR light propagation in the human finger, using Monte Carlo Modeling techniques. We consider light as photon packets which can undergo scattering, absorption, and internal reflections at interfaces in the tissue. We perform the simulation for multi layered tissue, incorporating optically homogeneous layers of skin, finger adipose tissue and the blood vessel. We first simulate the photon transport in rectangular slab geometry, followed by cylindrical geometry to more closely resemble the device functioning. The simulation calculates the fraction of photons detected by the photo-diode, which is compared to the actual device data for validation. There is an encouraging agreement between the predictions and the measurements.

8951-33, Session PMon

Switchable optical clearing window for monitoring development of diabetic angiopathy

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Diabetic angiopathy is the main complication of diabetes, which includes macroangiopathy arising as of ischemic brain disease or peripheral vascular occlusion, and microangiopathy arising as a result of destruction of capillaries, arterioles. In order to quantitatively evaluate the relationship between angiopathy and the development of diabetic, switchable optical clearing cranial window and dorsal skin window were applied by topical treatment of different optical clearing agents and saline on skull or skin based on our previous investigations. In this work, mice model of diabetes was made by alloxan. The glucose levels of blood were measured by a glucometer, and the cortical or dermal blood flow was monitored by laser speckle contrast imaging. With the switchable optical clearing windows, normal or diabetic cortical or dermal blood flow and their response to vasodilator or vasoconstrictor were investigated. The results show that the changes in cortical blood flow is more sensitive to glucose level than dermal blood flow, and the drugs induced changes in skin blood flow of diabetes mice are less than those of normal mice. The switchable windows not only enable to visualize the cortical or dermal blood vessels, and image blood flow at high resolution with laser speckle imaging technique, but also to track the same animal before or after made mouse model of diabetes.

8951-34, Session PMon

Pilot study to visualise and measure skin tissue oxygenation, erythema, total haemoglobin and melanin content using index maps in healthy controls

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We report on a method for analysing multispectral images of skin in vivo for the measurement and visualisation of four skin characteristics (skin tissue oxygenation, erythema, total haemoglobin and melanin content). Indices are used to characterise skin properties as they are less computationally intensive than regression techniques, making higher resolution images of larger areas possible. Multispectral data in the 500–720 nm range (7 nm bands, 5 nm apart) were collected with a Spectral TF VIS Imaging System (Channel Systems, Pinawa, MB, Canada) consisting of a liquid crystal tunable filter (LCTF) and CCD, illuminated with a broadband light source and analysed using custom software written in Matlab (Mathworks, Natick, Ma, USA). Four different indices were used to characterise skin tissue oxygenation, erythema, total haemoglobin and melanin content. Index values were calculated pixel-wise and combined to create index maps to visualise skin properties. Quantitative measurement of tissue oxygenation saturation was possible by calibrating the oxygenation index using a commercially available, calibrated oximeter (moorVMS-OXY, Moor Instruments, Devon, UK). Index maps were tested by arterial occlusion of the index finger with multispectral images taken before, during and after occlusion in a pilot study with 10 healthy controls. Index maps were found to be a suitable method for visualising skin properties and will be used in future studies to compare patients with systemic sclerosis and healthy controls.

8951-35, Session PMon

Quantitative measurement of biological substances in daily-life environment with the little-finger-size one-shot spectroscopic tomography

Akane Ishida, Sato Shun, Sho Nakada, Satoru Suzuki, Pradeep Abeygunawardhana, Kenji Wada, Akira Nishiyama, Ichirou Ishimaru, Kagawa Univ. (Japan)

In daily-life environment, the quantitative measurement of biological substances, such as the blood glucose level in the human skin, is strongly required to realize the non-invasive healthcare apparatus. Fourier-spectroscopic-tomography of the little-finger-size with high time-resolution and with the strong robustness for mechanical vibrations is proposed. The proposed method is a kind of near-common-path interferometer with spatial phase-shift method. We install the transmission-type relative-inclined phase-shifter on the optical Fourier transform plane of the infinity corrected optical system. The phase shifter are constructed with the cubic and the wedge prism to give the relative phase-shift spatially between each half-flux of the objective beams. The interferograms from each single-bright-points on an objective surface in a line are formed as fringe patterns on the 2-dimensional imaging array device. And because this proposed method is based on the imaging optics, only the emitted rays from the focal plane can contribute the forming of interferogram. Thus, the measurement plane can be limited onto the focal plane only. From the tomographic spectroscopy, only at a localized vessel area in human skins, we can get the pinpointed near-infrared spectroscopic data. And we can expect the improvement of the determination precision, because the Fourier spectroscopy can be acquired from the multiple intensity data in accordance with phase-shift value. From the statistical t-distribution point of view, the gradation of detector will be improved by square of sample number. We constructed the statistical model to assure the accuracy, and demonstrated the feasibility of the glucose sensor using the living rats.

8951-36, Session PMon

Wide-field spectroscopic imaging of biological-substance distributions on entire faces by measuring middle infrared lights emitted from human bodies itself

Yo Suzuki, Wei QI, Masaru Fujiwara, Hiroyuki Hiramatsu, Satoru Suzuki, Pradeep Abeygunawardhana, Kenji Wada, Akira Nishiyama, Ichiro Ishimaru, Kagawa Univ. (Japan)

We are aiming at the realization of the measurement technology for the biological-substance distributions, such as sebum, on entire faces at the daily-life environment. We proposed the imaging-type 2-dimensional Fourier spectroscopy that is the palm-size portable measurement apparatus and has the strong robustness for mechanical vibrations. And the proposed method can measure the wide-field 2-dimensional middle-infrared spectroscopic-imaging of radiation lights emitted from human bodies itself without light sources. In the proposed method, we install the phase-shifter, that can give an arbitrary phase shift for the half-flux of objective beams, at the optical Fourier transform plane of the infinity corrected optical system. The near-common-path interferometer, that is a phase-shift interferometer between objective beams, can be realized. In this proposed method, the emitted rays from each single-bright-points on measurement surfaces can interfere each other. Thus, even if the middle infrared-lights from human bodies are the spatially incoherent light, we can acquire the interferograms at each pixcels on

an imaging array device in accordance with the amount of phase shift as the 2-dimensional image-intensity changes. The conventional imaging FT-IR uses the phase-shifted beams with Michelson interferometer as a light source of a microscope. In principle, the narrow beam of phase-shifted lights only illuminate the narrow view-field whose width is around 1mm on a side. And for a kind of hyperspectral imaging, AOTF that is the variable narrow band-pass filter, can't apply to the middle infrared-light, because the light use efficiency is very low. We demonstrated the feasibility of sebum distributions on human faces.

8951-38, Session PMon

A demonstration of multiplex biosensing using whispering gallery mode microspheres positioned onto a microstructured optical fiber tip

Tess Reynolds, Alexandre François, The Univ. of Adelaide (Australia); Stephen J. Nicholls, South Australian Health and Medical Research Institute (Australia); Tanya M. Monro, The Univ. of Adelaide (Australia)

Exciting Whispering Gallery Modes (WGM) in a single dye-doped polymer microsphere positioned onto one of the MOF holes, next to the fiber core, offers many advantages such as automatically aligning the resonator with the fiber core, providing both the excitation and collection of the WGM modulated fluorescence emission from the resonator, and serves as a powerful dip sensing architecture. Since the refractive index symmetry around the resonator is broken by the presence of the MOF, it produces a polarization dependent enhancement of the radiative WGM emission which combined with stimulated emission results in a record effective Q factor above 104 for such resonator.

Here, we demonstrate that these advantages can be used for multiplexed sensing applications. The approach is to position multiple microspheres onto the tip of a single MOF. As a demonstration, two microspheres, doped with the same gain medium, were positioned next to each other onto the tip of a MOF. When excited below their lasing threshold, the individual resonances of each sphere cannot be distinguished. However, when operating the active microspheres above their lasing threshold, the resulting spectrum shows the de-convoluted resonance modes of the individual resonator. We show that this could also be used for tracking a specific biological interaction occurring onto one microsphere while using the other as a dynamic reference, correcting for temperature drift and non-specific binding. This concept could also be further extended to a larger number of microspheres to track multiple interactions against the one reference sphere.

8951-39, Session PMon

High-speed dual-wavelength optical polarimetry for glucose sensing

Daniel T. Grunden, Casey W. Pirnstill, Gerard L. Coté, Texas A&M Univ. (United States)

The significant rise in diabetic incidences over the past 50 years has increased research in non-invasive glucose monitoring techniques. While current commercial techniques have advanced significantly, their invasive nature often results in poor patient compliance. Thus, optical polarimetry in the anterior chamber of the eye has emerged as a potential technique to non-invasively measure glucose levels. Time varying corneal birefringence due to eye motion artifact confounds the optical signal ultimately limiting the polarimeter's accuracy to predict glucose concentrations. Previous work has shown that multispectral optical polarimetry has the potential for improving standard error in the presence of motion artifact. In this study, a high speed multi-spectral optical polarimetric approach has been developed and in vitro phantom studies were performed with glucose concentrations ranging from 0-600 mg/dL. Using a PID control system stability was reached in less than 10 msec, and glucose concentrations were predicted with a standard error less than 10 mg/dL. The results indicate that a high speed dual-wavelength polarimetric approach has the potential to be used for non-invasive glucose measurements through the anterior chamber of the eye.

8951-40, Session PMon

PEGylation of Concanavalin A to decrease nonspecific interactions in a fluorescent glucose sensor

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The ability of people with Diabetes to monitor and hence regulate blood sugar levels is limited by the common "finger-prick" test that provides intermittent, single point measurements. Toward the development of a continuous glucose monitoring (CGM) system, the lectin, Concanavalin A (ConA), has been utilized as a component in a Förster resonance energy transfer (FRET), competitive glucose binding assay. Recently, to avoid reversibility problems associated with ConA aggregation, a suitable competing ligand has been engineered. However, at physiological pH, native ConA exhibits a net negative charge that contributes to non-specific binding among ConA as well as with potential electrostatically charged, assay-delivery carriers thereby decreasing its ability to function as part of a glucose sensing assay. Therefore, to minimize non-specific binding and increase resistance to electrostatic surfaces for a delivery scheme, ConA was conjugated with monomethoxy-poly(ethylene glycol) (mPEG) (i.e. "PEGylation"). Previously shown to improve the solubility and stability of native ConA, fluorescently labeled ConA was PEGylated in order to decrease the electrostatic interactions with charged surfaces while maintaining affinity for the competing ligand. In this research, mPEG-succinimidyl succinate was covalently bound to free primary amines of lysine residues found near the surface of fluorescently labeled ConA (mPEG-SS-ConA). The binding activity of this modified ConA was characterized via fluorescence (intensity, lifetime, and anisotropy) and its electrostatic interactions were examined by introducing mPEG-SS-ConA to a layer-by-layer (LbL) assembly (poly(styrene sulfonate) (PSS)/ poly(allylamine hydrochloride) (PAH)).

8951-41, Session PMon

Multimodal assessment of spatial distribution of drug-tracer uptake by brain tissue after intra-arterial injections

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It is challenging to track the dynamics of intra-arterial (IA) drug administration, and traditional steady state assumptions based on intravenous (IV) administration are not valid. Site-specific delivery is affected by variations in vessel anatomy, drug streaming, and uneven drug-blood mixing. Optical methods for spatial mapping of drug deposition can assist in visualizing drug distributions and aid in the screening of potential drug candidates. We present a multimodal approach for the assessment of macroscopic drug distribution in postmortem tissue specimens and demonstrate its application in tracking IA liposome-dye conjugate delivery.

Liposomal nanoparticles were administered IA, and the Optical Pharmacokinetics (OP) method was used to track in vivo real-time drug concentrations as well as changes in oxy- and deoxyhemoglobin. Post-mortem cross-validation was performed with three optical techniques. Multispectral fluorescence imaging (MSFI) was performed on the freshly extracted brain; OP reflectance spectra were taken from select arterial distribution sites; and confocal microscopy (CM) was used to image serial sections. A stitching algorithm was used to combine individual CM frames to a wide-field image of each brain section. Fluorescence intensity values from MSFI were compared to the OP detected concentrations for select regions of the postmortem brain tissue. Pixel averaged fluorescence intensities were linearly proportional to OP detected concentrations of the same regions. MSFI fluorescence maps of serial sections correlated spatially to the unified CM images.

The results of this study suggest that valuable insight about a drug's spatial pharmacokinetics can be obtained by rapid optical methods. The aggregate use of these optical technologies yields a more comprehensive profile of drug deposition, helping to streamline the screening process for IA drug delivery.

8951-42, Session PMon

Developing strategies to enhance loading efficiency of erythrosensors

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For diabetics, continuous glucose monitoring and the resulting tighter control of glucose levels ameliorate serious complications from hypoglycemia and hyperglycemia. Diabetics measure their blood glucose levels multiple times a day by finger pricks, or use implantable monitoring devices. Still, glucose and other analytes in the blood fluctuate throughout the day and the current monitoring methods are invasive, immunogenic, and/or present biodegradation problems. Using carrier erythrocytes loaded with a fluorescent sensor, we seek to develop a biodegradable, efficient, and potentially cost effective method to continuously sense blood analytes. We aim to reintroduce sensor-loaded erythrocytes to the bloodstream and conserve the erythrocytes lifetime of 120 days in the circulatory system. Here, we compare the efficiency of two loading techniques: hypotonic dilution and electroporation. Hypotonic dilution employs hypotonic buffer to create transient pores in the erythrocyte membrane, allowing dye entrance and a hypertonic buffer to restore tonicity. Electroporation relies on controlled electrical pulses that results in reversible pores formation to allow cargo entrance, follow by a 37 incubation to reseal. As part of the cellular characterization of loaded erythrocytes, we focus on measuring volume, hemoglobin content, osmotic fragility and stiffness. Cell recovery, loading efficiency and cargo release measurements render optimal loading conditions. The detected fluorescent signal from sensor-loaded erythrocytes can be translated into a direct measurement of analyte levels in the blood stream. The development of a suitable protocol to engineer carrier erythrocytes has profound and lasting implications in the erythrocytes lifespan and sensing capabilities.

8951-43, Session PMon

Biomarkers of chronic kidney disease in the urine of diabetic/hypertensive patients by means of Raman spectroscopy

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Diabetes mellitus (DM) and arterial hypertension (AH) are common diseases that, if untreated, predispose the patient to renal failure. This study aimed to evaluate possible biomarkers in the urine of patients with DM and AH capable to predict the chronic renal disease, by means of Raman spectroscopy. Urines were obtained from patients with DM and AH, and separated into four groups: no symptoms of diseases related to DM and AH (G1), with low clinical complications (G2), with severe clinical complications (G3), and with chronic kidney disease (G4) arised from DM and AH. It has been used a dispersive Raman spectrometer (830nm, 300mW, 20s accumulation). In the spectra of urine it was identified Raman peaks at 680cm-1 (creatinine), 1004cm-1 (urea) and 1128cm-1 (glucose). The results revealed that G2, G3 and G4 presented the creatinine peak with lower intensity than G1 ($p<0.001$). It was observed that G2, G3 and G4 showed lower intensity of the urea peak compared to G1 ($p<0.001$) and G4 showed lower intensity compared to G2 and G3 ($p<0.001$). Despite not significant, the glucose peak showed lower intensity in G1 when compared to the other groups. A model for classification of groups according to clinical criteria, using Sparse Multinomial Logistic Regression, taking as inputs the intensities of creatine, urea and glucose peaks allowed correct classification of 88.9% for G1, 36.8% for G2, 43.8% for G3 and 84.2% for G4. These results demonstrated the possibility of obtaining diagnostic information for complications of kidney disease associated to DM and AH.

8951-1, Session 1

A simple and robust optical scheme for self-mixing low-coherence flowmeters

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Laser Doppler velocimetry (LDV) is a well-known and widely used measurement technique.

However, when the fluid is turbid or the duct is buried in a turbid medium, e.g. a blood vessel, multiple-scattering regime sets in, thus techniques based on coherent sources are known to suffer great uncertainty.

Unfortunately, this is a quite typical situation in many biological tissues.

Low-coherence illumination can be used to overcome this limitation. As an example, Doppler optical coherence tomography (DOCT) allows to measure flow-velocity profiles either in the case of highly scattering fluids or when the duct is buried in a turbid medium.

Despite the advantages offered by LDV and DOCT systems, some drawbacks, i.e. complexity and costs, limit the field of applications.

System complexity and cost can be significantly reduced by exploiting the self-mixing (SM) approach. The SM technique requires that a portionof the light emitted by the source is backscattered from the moving scatterers and re-enters the source cavity where it causes measurable changes in emitted power. The resulting output-power fluctuations are measured by using the back-facet monitor photodiode.

However classical optical scheme based on a low-coherence source requires a reference arm to set the measuring region, this increases the complexity of the system also compromising its robustness.

In this paper we propose an optical scheme that exploits the reflection from the inner wall of the duct as a reference arm. Such solution highly simplify the optics avoiding problems related to movements of the reference arm and/or measurand.

8951-2, Session 1

Photoplethysmography beyond perfusion and oxygenation monitoring: pulse wave analysis for hepatic graft monitoring

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Photoplethysmography is a technique widely used in monitoring perfusion and blood oxygen saturation based on the amplitude of the pulsatile signal at one or multiple wavelengths. However, the pulsatile signal carries in its waveform a substantial amount of information about the mechanical properties of the tissue and vasculature under investigation that is still yet to be utilized to its full potential. In this work, we present the feasibility of pulse wave analysis for the application of monitoring hepatic implants and diagnosing graft complications. In particular, we demonstrate the utility of computing the slope of the pulse during the diastole phase to assess the location of vascular complications when they take place. This hypothesis was tested in a series of in vitro experiments using a PDMS-based phantom mimicking the optical and mechanical properties of the portal vein. The emptying time of the vessel increased from 305 ms to 515 ms when an occlusion was induced downstream from the phantom. However, in the case of upstream occlusions, the emptying time remained constant. In both cases, a decrease in the amplitude of the pulse was recorded indicating the drop in flow levels. In addition, we show that quantifying the emptying time of the vasculature under investigation can be used to assess its compliance. The emptying time decreased from 305 ms for phantoms with compliance of 15 KPa to 195 ms for phantoms with compliance of 100 KPa. These compliance levels mimic those seen for normal and fibrotic hepatic tissue respectively.

8951-3, Session 1

Optical monitoring of skeletal muscle oxygenation and hemodynamics during exercise: a novel approach in exercise science

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Fitness monitoring is an essential step required to control and improve body performance and to prevent overuse injury and exercise-induced muscle fatigue. Current gold standard of fitness monitoring is limited to heart rate (HR) monitoring, a method that gives a general index of whole body function with inherent restrictions that make it a limited and unreliable method in many situations.

Using a wireless near infrared spectroscopy (NIRS) device we have shown that direct real-time monitoring of limb muscle hemodynamics and oxygenation during exercise and recovery period can provide more in depth information on exercise intensity and muscle fitness comparing HR monitoring during exercise.

Ten healthy adult subjects were recruited to run for 60 minutes within their aerobic capacity range - 75% of their maximum heart rate (HR-max) - using a HR monitoring system (Polar-610) while changes in their calf muscle oxygenated hemoglobin (O2Hb), deoxygenated hemoglobin (HHb), total hemoglobin (THb) and tissue oxygenation index (TOI) were monitored by a NIRS device (OxiTor-M2) in spatially resolved configuration during exercise and recovery.

Our data showed that while HR and therefore exercise intensity were constant, muscle hemodynamics and oxygenation changed in a specific pattern during different stages of running period. In general blood flow and level of oxygenation showed a gradual increase within exercising calf muscles during a 60-minute aerobic running period ($P<0.05$).

This observation suggests wireless optical monitoring of muscle metabolism as a novel noninvasive method for monitoring exercise intensity and muscle condition during exercise and functional rehabilitation.

8951-4, Session 1

Real-time imaging the blood flow using laser speckle: from basic to preclinic studies

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Laser speckle contrast imaging has been widely attempted to detect the blood flow changes in brain, skin, retina, arthrosis, liver and so on with the advantages of high spatial and temporal resolution, full-field imaging without scanning. It provides important tool for investigating the physiology and pathology of biological tissues, and evaluating the clinical diseases. However, several problems still need to be investigated either in methodology or application to improve its performance and promote its clinic applications. Recent technical progresses of laser speckle contrast imaging of blood flow on improving imaging speed, spatial resolution, signal to noise ratio, statistics accuracy, imaging depth and so on were discussed. The applications of this technique on basic researches and clinical diagnostics and therapies, such as photodynamic therapy of Port-wine stains, estimation of the depth of burns, perfusion evaluation of diabetic foot and so on were also demonstrated.

8951-5, Session 1

High-resolution coherence domain depth-resolved nailfold capillaroscopy based on correlation mapping optical coherence tomography

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In this paper we demonstrate a novel application of correlation mapping optical coherence tomography (cm-OCT) for volumetric nailfold capillaroscopy (NFC). NFC is a widely used non-invasive diagnostic method to analyze capillary morphology and microvascular abnormalities of nailfold area for a range of disease conditions. However, the conventional NFC is incapable of providing volumetric imaging, when volumetric quantitative microangiopathic parameters such as plexus morphology, capillary density, and morphologic anomalies of the end row loops most critical. cm-OCT is a recently developed well established coherence domain magnitude based angiographic modality, which takes advantage of the time-varying speckle effect, which is normally dominant in the vicinity of vascular regions compared to static tissue region. It utilizes the correlation coefficient as a direct measurement of decorrelation between two adjacent B-frames to enhance the visibility of depth-resolved microcirculation.

8951-6, Session 2

Modeling the optical coupling across the anterior chamber of the eye towards polarimetric glucose sensing

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Millions of people worldwide are affected by diabetes. While glucose sensing technology has come a long way over the past several decades, the current commercially available techniques are still invasive, often leading to poor patient compliance. To minimize invasiveness, focus has been placed on optical techniques to ascertain blood glucose concentrations. Optical polarimetry has shown promise and progress as a viable technique for glucose sensing. Recent developments

in polarimetric glucose sensing have been focused on overcoming time varying birefringence due to motion artifacts. Beyond corneal birefringence, the next hurdle toward making this approach viable is the ability to couple polarized light across the eye's anterior chamber. The eye is ideally suited to couple light to the retina. The index mismatch between the cornea and air is partially responsible for the beam bending toward the retina and, while good for vision, it complicates our ability to couple light across the anterior chamber without index matching for polarimetric glucose monitoring. In this report, we have developed polarized light ray tracing models for evaluating and optimizing various optical coupling designs that allow for non-index matched light coupling across the aqueous humor. The ray tracing models were developed for a dual wavelength system and evaluate refraction, diattenuation, reflection, and polarization changes at each optical interface within the setup.

8951-7, Session 2

Influence of spectral bandwidth limitations of tuneable external-cavity based quantum cascade laser systems for clinical biofluid analysis

Herbert M. Heise, Thorsten Vahlsing, Fachhochschule Südwestfalen (Germany)

In many publications, infrared spectroscopy has shown its excellent performance in quantitative multi-analyte analysis of biofluids. However, its applicability for point-of-care use or bed-side monitoring has been limited due to the still bulky size of conventional FTIR-spectrometers compared to single-analyte biosensors. With the recent development of room temperature operated quantum cascade lasers, utilising an external cavity for broad tuneability, infrared laser spectroscopy became feasible for quantitative biofluid analysis. For an optimized sample pathlength, the noise performance of EC-QCL based spectrometer systems has been shown to be at least equal to state of the art FTIR-instrumentation. However, an available spectral bandwidth of about 200 cm-1 for a one-laser system is much smaller compared to the full mid-infrared range accessible with FTIR-devices.

For this work, we applied a bandwidth constraint to previous FTIR-studies on spectroscopic assays of blood plasma, dialysates of biofluids and aqueous solutions of blood components including common interfering drugs. For the clinically important blood glucose with its substantial band structure in the fingerprint region, there was no impairment, provided that collinear spectral interferents such as hydroxyethyl starch used as plasma expander were absent. Other analytes of interest, such as urea, showed a less favourable characteristic. Although access to the main urea bands, which are overlapping with strong water absorption, should be feasible with the high available power of an EC-QCL, the necessary tuneability is about 400 cm-1 for optimum performance and a second laser will be needed for a simultaneous glucose assay.

8951-8, Session 2

Blood analyte sensing using fluorescent dye-loaded red blood cells

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Measurement of blood analytes provides crucial information about a patient's health. Some such analytes, such as glucose in the case of diabetes, require long-term or near-continuous monitoring for proper disease management. However, current monitoring techniques are far from ideal: multiple-per-day finger stick tests are inconvenient and painful for the patient; implantable sensors have short functional life spans (i.e., 3-7 days). Due to analyte transporters on red blood cell (RBC) membranes that equilibrate intracellular and extracellular analyte levels, RBCs serve as an attractive alternative for encapsulating analyte sensors. Once reintroduced to the blood stream, the functionalized RBCs may continue to live for the remainder of their life span (120 days for humans). They are biodegradable and biocompatible, thereby eliminating the immune system response common for many implanted devices. The proposed sensing system utilizes the ability of the RBCs to swell in response to a decrease in the osmolarity of the extracellular solution. Just before lysis, they develop small pores on the scale of tens of nanometers. While at low temperature, analyte-sensitive dyes in the extracellular solution diffuse into the perforated RBCs and become entrapped upon restoration of temperature and osmolarity. Since the fluorescent signal from the entrapped dye reports on changes in the analyte level of the extracellular solution via the RBC transporters, interactions between the RBCs and the dye are critical to the efficacy of this technique. In this work, we study these interactions and their impact on the RBC sensing platform.

8951-9, Session 2

ConA-based glucose sensing using the long-lifetime azadioxatriangulenium fluorophore

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Fluorescent glucose sensing technologies have been identified as possible alternatives to current continuous glucose monitoring approaches. We have recently shown an improved glucose sensing assay based on the lectin Concanavalin A (ConA) by using a competing ligand with the core trimannoside of N-linked glycans. Work here is a preliminary attempt to use a variation of this new competing ligand in a ConA-based glucose sensing assay to measure glucose concentrations in vitro using the cell culture media (DMEM) as a physiologically relevant solution. Through 3D excitation/emission scans of the media, it is clear there are at least 3 fluorescent and/or absorbing species in the visible range. To minimize the effect that these optical interferents have on the functionality of the fluorescent glucose assay, we use the red-emitting, long-lifetime azadioxatriangulenium (ADOTA) dye [1]. Using an ex/em of 503/600 nm with a 1 uM solution of ADOTA in DMEM buffer, the long-lifetime of ADOTA is easily distinguishable (~19 ns) from the background signal from DMEM (~4 ns). The fluorescent competing ligand is synthesized by conjugating ADOTA-NHS to ovalbumin via traditional amine modification. Ovalbumin is a glycoprotein with a single asparagine residue capable of being glycated with a core trimannoside bearing N-linked glycan and has a similar isoelectric point to ConA. Thus, binding between ConA and ovalbumin should be monovalent and sugar specific. After conjugation of ADOTA, affinity chromatography is used to retain only the glycated portion of the ADOTA-ovalbumin. The equilibrium binding between the glycated ADOTA-ovalbumin and unlabeled ConA is shown via fluorescence anisotropy.

[1] Azadioxatriangulenium: a long fluorescence lifetime fluorophore for large biomolecule binding assay. Thomas Just Sørensen et al 2013 Methods Appl. Fluoresc. 1 025001.

8951-10, Session 3

Blood cell diagnostics by a chip lensless microscope

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Technologien e.V. (Germany); Guenter Mayer, Leibniz-Institute of Photonic Technology (Germany)

Simple miniaturized holographic microscopes are used more and more for health care and biological applications. We present the digital inline holographic microscopy for blood cell counting. The microscope unit contains a pinhole chip for illumination, a micro-fluidic chip, and a CCD-chip for detection.

The design and the dimension conditions are discussed from the point of blood cell diagnostics as well as from the point of lensless holographic microscopy. For illumination a modulated laser with a center wavelength of 661 nm is used. An adaption of the degree of coherence into the range of 50 µm essentially improves the image quality. A pinhole with a diameter of 800 nm diffracts the laser light to a spherical wave front. The numerical aperture up to .85 enables a spatial resolution below 1µm. Therefore different refractivities along the optical path through the microfluidic chip have to implicate in the reconstruction process.

A single hologram delivers a stack of images for all z-positions in the micro-fluidic channel and is the basic for counting of flowing cells. A video of flowing blood cells is presented which will be used in clinical diagnostics.

8951-11, Session 3

Preliminary measurement results of an optical cavity based biosensor using chained differential detection

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We report an optical cavity based biosensor using a novel chained differential detection method. A three laser diode sensing mechanism provides multiplexing capability and is used to enhance the responsivity and increase the dynamic range using differential detection and a sliding window approach. The laser wavelengths are evenly spaced and the cavity width chosen so that the optical intensities of two lasers change along opposite and monotonic slopes upon the immobilization of target biomarkers. The way to enhance the sensitivity through a differential calculation using these two intensities is two-fold: (1) Due to the opposing and monotonic slopes, the corresponding slope of the differential value changes faster than optical intensities. (2) Since all three wavelengths of light propagate through the same path, some uncontrollable variations along the path will be effectively canceled out by taking the differential calculation, reducing the noise due to such variations. By cycling through the pair of lasers used in the differential calculation, a dynamic range of 1 ?m is achieved. The average responsivity (i.e. slope of the differential value with respect to the sensing layer thickness change) has been demonstrated via simulation to be 0.01082/nm. The high sensitivity of the sensor coupled with its large dynamic range makes it well suited for a multiplexed biosensor where a variety of biomolecules of different sizes at different concentrations must be accurately measured simultaneously.

In this presentation, we will discuss the simulation results, fabrication procedures, detection system, and preliminary measurement results.

8951-12, Session 3

Fluorescent imaging over an ultralarge field-of-view of 532 cm^2 using a flatbed scanner

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Fluorescent imaging over a large field-of-view is usually done by scanning optical microscopes and requires the time consuming process of capturing and stitching several thousand images due to the limited field-of-view of objective lenses. Here we introduce a portable and cost-effective fluorescent imaging method that is capable of detecting fluorescent micro objects over an ultra large field-of-view of 19 cm ? 28 cm, i.e., 532 cm^2. The core component in our design is a conventional flatbed scanner, where we modified both the hardware and software components, added a custom-designed absorbing emission filter, and a computer controlled two dimensional LED array for fluorescent excitation. We took advantage of the relatively low numerical aperture of the gradient index lens array inside the scanner head by using a high incidence angle fluorescent excitation, thus assuring that the excitation light does not directly reach the sensor. We also reprogrammed the driver of the scanner device to maximize the sensitivity, exposure time and gain for fluorescent detection of micro-objects. This high-throughput fluorescent imaging system could be quite useful for point-of-care cytometry applications and rare cell research by allowing rapid screening of substantial volumes of optically dense media. For example, by using this modified scanner platform in conjunction with a custom made microfluidic chip, this ultra large field of view (532 cm^2) allows us to screen for fluorescent micro-objects inside more than 2.2 mL of undiluted whole blood within 5 minutes.

8951-13, Session 3

Label-free molecular sensing by SERS on nanoporous gold substrates

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Label-free, “on-chip” sensing of a broad range of physiologically-relevant biomolecules such as metabolic analytes and nucleotides would potentially contribute to new point-of-care diagnostic sensors. Surface-enhanced Raman spectroscopy (SERS) has been widely used for high-sensitivity molecular detection and identification, thanks to the localized surface plasmon resonance (LSPR) effect. Because LSPR is a near-field phenomenon and decays rapidly with increased separation distance between the molecule and the nanostructure, SERS signal primarily arises from the molecules residing within a few nanometers of the nanostructured surface. Therefore, it is advantageous for a SERS substrate to have a large surface-to-volume ratio from the standpoint of high-density “hot spots”, as well as optical collection efficiency in point-of-care sensors.

Recently, we have utilized the intrinsic plasmonic properties in nanoporous gold thin films for molecular sensing. Specifically, we have developed a SERS substrate by shaping nanoporous gold thin films into monolithic submicron disks, called nanoporous gold (NPG) disks. NPG disks provides an effective surface area >10X larger than its geometrical area and a SERS enhancement factor larger than 100 million [1]. Our approach features hybrid fabrication by combining top-down planar large-area sputter etching and bottom-up atomic self assembly during dealloying. The resulted structure is thus hierarchical with the external disk shape and the internal porous network. We have selected 785 nm as the laser excitation wavelength and benzenethiol (BT) molecules as the SERS marker since the absence of a BT absorption peak near 785 nm minimizes the ambiguity presented by resonant Raman scattering, while the ability of BT to form self-assembled monolayers (SAMs) enables the number of molecules on individual NPG disks to be quantified. Additionally, the SERS activity at 785 nm laser excitation has critical significance for deep tissue penetration in any potential biomedical applications.

NPG disk substrates have been employed in SERS label-free detection of biomolecules at physiologically relevant concentrations. We have demonstrated robust SERS measurement of neurotransmitter such as dopamine and metabolic analytes such as urea in low micromolar range, and un-labeled nucleotides at ~10 nanomolar, all with microliter sample volume.

References

[1] J. Qi, P. Motwani, M. Gheewala, C. Brennan, JC Wolfe and W.-C. Shih, “Surface-enhanced Raman spectroscopy with monolithic nanoporous gold disk substrates,” Nanoscale, 5, 4105-4109 (2013).

8951-14, Session 3

Rapid identification of bacterial resistance to Ciprofloxacin using surface-enhanced Raman spectroscopy

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Ciprofloxacin is a second generation fluoroquinolone which kills bacteria by interfering with the mechanism of DNA rewinding after replication. It has a wide spectrum of activity against both gram negative and gram positive bacteria and is, therefore, one of the most effective antibiotics used for treatment of bacterial infections, including those of the urinary tract. Due to its effectiveness it is the fifth most prescribed antibacterial in the US. Current methods of bacterial infection diagnosis and antibiogram can take as much as 48 hrs, due to the requirement for two sequential overnight cultures. As a result, physicians prescribe ciprofloxacin and other broad spectrum antibiotics before obtaining antibiotic sensitivity results. This leads to ineffective treatments and chronic infections but most importantly to more strains becoming resistant to ciprofloxacin and other commonly used antibiotics. In order to protect such useful antibiotics from becoming ineffective, a method that provides rapid diagnosis of an infection as well as an antibiogram, in 4 hours or less, was developed. This method determines bacterial susceptibility to Ciprofloxacin using Surface Enhanced Raman Spectroscopy (SERS) with silver nanoparticles. SERS spectra of five species of gram negative bacteria, namely Escherichia coli, Proteus spp., Klebsiella spp., Enterobacter spp., and Citrobacter spp. were obtained after a 4 hour exposure to Ciprofloxacin. Spectral analysis revealed clear separation between bacterial samples which were sensitive to Ciprofloxacin and samples which were resistant. With the enhancement provided by SERS, the technique could be applied directly to urine or blood samples, bypassing the need for overnight cultures.

8951-15, Session 4

Cost-effective fluorescence microscope for point of care read out of bead-based assays

Alessandra J. Forcucci, Ina P. Pavlova, Michal Pawlowski, Rebecca R. Richards-Kortum, Tomasz S. Tkaczyk, Rice Univ. (United States)

A number of new platforms have been developed for multiplexed bioassays that rely on imaging targeted fluorescent beads labeled with different fluorescent dyes (e.g. Luminex). These systems typically rely on macroscale readers that are too bulky and expensive for use at the point-of-care. We developed a compact fluorescence microscope that can be used as a detector for mutiplexed, bead-based assays that would reduce cost and support point-of-care applications. In Luminex's MagPix system, a family of fluorescent microspheres serves as a spectral identifier and solid surface to build analyte-specific assays. Our cost-effective modular microscope can perform optical read out in low-resource point-of-care settings for bead-based assays, particularly Luminex's MagPix system.

The microscope is composed of 3D-printed plastic several modules that can be modified depending on the application. The Illumination module houses the LED light sources (505nm and 617nm), a collecting lens, a condenser lens and two excitation filters (615/20nm and 510/20nm) to excite multiple dyes. The sample chamber is compatible with standard microscope slides. The objective module houses a 4x, 0.25 NA infinity-corrected microscope objective with manual focus adjustment capability. The emission filter module houses three filters: 593/40 nm, 661/20 nm, and 720/13 nm for the reporter, classification 1, and classification 2 channels, respectively. The tube lens module houses an infinity-corrected tube lens with manual focus adjustment capability. The detector module contains a USB compatible CMOS detector, optionally available with GSM transmitter. A series of experiments were conducted in which we validated the ability of our device against the Magpix to classify three different bead types.

8951-16, Session 4

Stick-on microscope for smartphones

Woei Ming Lee, Australian National Univ. (Australia)

Microscopy has transformed from a past-time hobby in the 17th century to blooming industry. Global Industry Analysts Inc. forecasted that the microscopy market will be worth more than US$ 3.98 billion dollars by 2017. While the optical technology behind light microscopy have seen little change over the last few centuries, the usage of 21st century digital imaging technology in microscopy system has rapidly changed how microscope data are captured and analysed. The straightforward addition of standard miniature lens elements (aspheric, graded lens) onto smartphone cameras in commercial mobile microscopes, such as Keeploop, Handyscope, Cellscope, are challenging conventional light microscopy. However, for a mobile microscope to be truly suitable for routine and rugged use, the existing materials used to make miniature lens do not possess enough physical resilience. Furthermore, they add significant cost, weight and size to existing smartphones.

Here I proposed a new form of microscope based on transparent elastomer materials. My current microscope design can be tailored to the size of any smartphone cameras and also exhibit micrometer resolution imaging . In contrast to existing mobile microscope device, my existing device costs a mere USD$ 2.002 (lenses ~ USD$0.002, light emitting diode ~USD $2) and weighs ~0.01. The entire microscope is around two times smaller than any existing mobile microscope designs (~5 mm thickness). This new form of ultra-lightweight and low cost microscope device that is seamlessly integrated into existing smartphone cameras could set a new trend in mobile microscopy.

8951-17, Session 4

Simple microscopy on a smartphone for skin melanin diagnosis

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Fair-skinned viewed as a symbol of beauty for Asian women, they do a lot of effort to whiten their skin. Therefore, an easy way to monitor the status of skin is a benefit for daily personal beauty and healthcare. We demonstrate a simple and low cost design microscopy on a smartphone, instead of ordinary dermatoscope for skin detection. This imaging platform can be mechanically attached to the camera unit of the smartphone where the samples can be touched by a lens and are illuminated by a simple light-emitting diode (LED). This incoherent LED light is then scattered from each micro-object to coherently interfere with the background light, creating the hologram of each object on the detector of the phone. Furthermore, this device can also monitor the melanin status in the skin and such high throughput and miniaturized imaging devices can provide a complementary toolset for telemedicine applications and point-of-care diagnostics through smartphone apps and the internet. We show the performance of this smartphone microscope by clearly 3D imaging various sized micro-particles, as well as skin pores, moles even the melanin under skin which is also ideal for affordable point-of-care devices aiming at resource-limited places.

8951-18, Session 4

Dual-wavelength excitation to reduce background fluorescence for fluorescence spectroscopic quantitation of erythrocyte zinc protoporphyrin-IX and protoporphyrin-IX from whole blood and oral mucosa

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Erythrocyte zinc protoporphyrin-IX (ZnPP) and protoporphyrin-IX (PPIX) accumulate in a variety of disorders that restrict or disrupt the biosynthesis of heme, including iron deficiency and various porphyrias. We describe a reagent-free spectroscopic method based on dual-wavelength excitation that can measure simultaneously both ZnPP and PPIX fluorescence from unwashed whole blood while virtually eliminating background fluorescence. We further aim to quantify ZnPP and PPIX non-invasively from the intact oral mucosa using dual-wavelength excitation to reduce the strong tissue background fluorescence while retaining the faint porphyrin fluorescence signal originating from erythrocytes.

Fluorescence spectroscopic measurements were made on 35 diluted EDTA blood samples using a custom front-face fluorometer. The difference spectrum between fluorescence at 425nm and 407nm excitation effectively eliminated background autofluorescence while retaining the characteristic porphyrin peaks. These peaks were evaluated quantitatively and the results compared to a reference HPLC-kit method. A modified instrument using a single 1000µm fiber for light delivery and detection was used to record fluorescence spectra from oral mucosa.

For blood measurements, the ZnPP and PPIX fluorescence intensities from the difference spectra correlated well with the reference method (ZnPP: Spearman's rho rs=0.943, p<0.0001; PPIX: rs=0.959, p<0.0001). In difference spectra from oral mucosa, background fluorescence was reduced significantly, while porphyrin signals remained observable.

The dual-wavelength excitation method evaluates quantitatively the ZnPP/heme and PPIX/heme ratios from unwashed whole blood, simplifying clinical laboratory measurements. The difference technique reduces the background fluorescence from measurements on oral mucosa, allowing for future non-invasive quantitation of erythrocyte ZnPP and PPIX.

8951-19, Session 4

Point-of-care optical tool to detect early stage of hemorrhage and shock

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There is a critical unmet clinical need for a device that can monitor and predict the onset of shock: hemorrhagic shock or bleeding to death, septic shock or systemic infection, and cardiogenic shock or blood flow and tissue oxygenation impairment due to heart attack. Together these represent ~141 M patients per year. We have developed a monitor for shock based on measuring blood flow in peripheral (skin) capillary beds using diffuse correlation spectroscopy, a form of dynamic light scattering, and have demonstrated proof-of-principle both in pigs and humans. Our results demonstrate that skin blood flow measurement either alone or in conjunction with other hemodynamic properties such as heart rate variability, pulse pressure variability, and tissue oxygenation can meet this unmet need in a small self-contained patch-like device in conjunction with a hand-held processing unit. In this paper we describe and discuss the experimental work and the multivariate statistical analysis performed to demonstrate proof-of-principle of the concept.

8951-20, Session 5

Non-model-based approach for determining depth and concentration of deep fluorescent lesions in turbid media

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Many biological fluorescent markers are being developed in order to label tissue of interest, such as cancer. When this is used in conjunction with surgery, it provides visual feedback to the surgeon as to the location of the tumor. Wide-field detection of subsurface fluorescence presents interesting challenges since the detected signal is a function of depth, concentration and size. Several attempts have been made to decouple these factors in order to quantify the depth and/or the concentration of the fluorescent lesion, but most rely on obtaining bulk optical properties of the medium. This work presents a non-model based approach to determining depth by using the diffuse reflectance spectrum of the medium without needing to calculate the optical properties. Since there is variation in optical properties with wavelength, it has been shown that the logarithm of the ratio of the fluorescence emission at two discrete wavelengths changes linearly with the depth of the fluorescence. Sampling the medium with white light and taking the ratio of the diffuse reflectance at the same two wavelengths as the fluorescence ratio provides a close estimate of the rate of change of the fluorescence ratio with depth. This technique has been used in phantoms with Alexa Fluor 647 to predict depth up to a signal-to-background ratio of 10 (about 5 mm) with an accuracy of 1 mm in a wide-field geometry. This work also presents preliminary in vivo evaluation of the technique using ALA-induced fluorescence in a glioma model.

8951-22, Session 5

In vivo and in vitro hyperspectral imaging of cervical neoplasia

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Cervical cancer is a prevalent disease in many developing countries. Colposcopy is the most common approach for screening cervical intraepithelial neoplasia (CIN). However, its clinical efficacy heavily relies on the examiner's experience. Spectroscopy has the potential to be an effective method in cervical neoplasia diagnosis. In this paper, we have proposed an effective hyperspectral imaging system using visible and near-infrared wavelengths region to detect and quantify cervical neoplasia. A hyperspectral imager is used to collect reflectance images of entire cervix under xenon lamp illumination before acetic-acid application, and then standard colposcopy examination and cervical

samplings from normal and abnormal sites in different quadrants are performed. The reflectance data are calibrated and the specular reflection on the raw cervical images is eliminated. The hyperspectral signals are extracted and evaluated in different sites, correspondingly the cervical images are classified into several separate parts accounting for different stages of cervical intraepithelial neoplasia. The analysis results are compared with detailed histopathological results to assess accuracy and efficacy of the method.

8951-24, Session 5

Measurement of the human esophageal cancer in an early stage with Raman spectroscopy

Yasuhiro Maeda, Mika Ishigaki, Akinori Taketani, Bibin B. Andriana, Kwansei Gakuin Univ. (Japan); Ryu Ishihara, Osaka Medical Ctr. for Cancer and Cardiovascular Diseases (Japan); Hidetoshi Sato, Kwansei Gakuin Univ. (Japan)

The esophageal cancer has a tendency to transfer to another part of the body and the surgical operation itself sometimes gives high risk in vital function because many delicate organs exist near the esophagus. So the esophageal cancer is a disease with a high mortality. So, the detection of the esophageal cancer in the early stage is essentially expected for the better outcome for the therapy. In order to lead a higher survival rate five years after the cancer's treatment, the investigation of the diagnosis methods or techniques of the cancer in an early stage and support the therapy. Raman spectroscopy is one of the most powerful techniques for the purpose. In this study, we performed the ex vivo experiments to obtain normal and early-stage tumor (stage-0) human esophageal sample by using Raman spectroscopy. The Raman spectra are corrected by the Raman spectrometer which consists of a diode laser with the wavelength of 785 nm, Raman probe with 600-um-diameter and spectrometer with the resolution of 1 cm^-1. The principal component analysis (PCA) is performed after collection of spectra to recognize which materials changed in normal part and cancerous pert. After that, the linear discriminant analysis (LDA) is performed to predict the tissue type. The result of PCA indicates that the tumor tissue is associated with a decrease in tryptophan concentration. Furthermore, we can predict the tissue type with 80% accuracy by LDA which model is made by tryptophan bands.

8951-25, Session 5

Raman Endoscopy for Real Time Monitoring of Anticancer Drug Treatment in Colorectal Tumors of Live Model Mice

Akinori Taketani, Mika Ishigaki, Bibin B. Andriana, Hidetoshi Sato, Kwansei Gakuin Univ. (Japan)

The aim of the present study is to evaluate the capability of a miniaturized Raman endoscope (mRE) system to monitor the advancement of colorectal tumors in model mice as a method that is noninvasive to the tumor itself. The endoscope is narrow enough to observe the inside of the mouse colon in a way that is semi-noninvasive to the animal. Raman spectroscopy allows obtaining information about molecular concentrations and the structural composition of tumor tissues in situ in a totally noninvasive manner. However, the mRE system allowed the visualization and Raman spectral measurement of any targeted point within the colorectal tumor in model mice under anesthesia, without damaging the tissue (i.e., noninvasively). Continuous monitoring of the same tumor allowed the observation of alterations in its molecular composition and size, along with its advancement. The Raman spectrum of before and after the anticancer (5-FU)-treated colorectal cancer model mice are also measured for monitoring the effect of anticancer drugs in situ. Our Raman system equipped with a miniaturized endoscope and BHRP can simultaneously obtain spectral and visual information on the same tumor in a single mouse through continued measurements for a several weeks. The tumor lesion was discriminated from normal tissues of the control mouse with an accuracy of 86.8%. The Raman spectrum of before and after the anticancer-treated model mice are differentiable?The Raman analysis suggested that it was not cured but supposedly transformed to another tumor type.

8951-27, Session 6

Multiwavelength pulse plethysmography for real-time drug delivery monitoring

Pratik Adhikari, Isidro B. Magaña, Patrick D. O'Neal, Louisiana Tech Univ. (United States)

A novel multi wavelength photo plethysmograph, initially utilized to quantify circulating gold nanoparticles, has previously demonstrated the potential to enhance therapeutic treatment predictability as pharmacokinetic metrics are provided throughout the delivery phase in near real-time. This report presents a modified prototype that can be used to assess the real time bioavailability of other types of intravenously delivered optically-absorbing nanoparticles and drugs. Initial experiments established the upper (~8.6 OD) and lower (0.3 OD) limits of detection for intravenously injected gold nanorods in a murine model. The drugs currently under investigation include cancer therapeutics such as Doxorubicin/Doxil (absorption peak ~460 nm, fluorescent emission peak ~600 nm), anti-malarial quinine (absorption peak ~350 nm, fluorescent emission peak ~460nm), and an agent used in photodynamic therapy. At the working wavelengths for each compound, the effects of scattering and the absorbance of endogenous elements are reported via a mathematical estimation of the tissue optical properties and resulting penetration depth. This report demonstrates the capabilities of the prototype by estimating the concentration of the agents in the pulsatile blood by absorbance spectrometry, and includes estimates of oxygen saturation typically obtained via pulse oximetry. The detectability of the exogenous agents are reported in terms of the upper and lower detection limits of the device for each compound.

8951-28, Session 6

Fiber-enhanced Raman sensing of pharmaceutical drugs

Torsten Frosch, Di Yan, Juergen Popp, Institut für Photonische Technologien e.V. (Germany)

We present the technique fiber enhanced UV resonance Raman spectroscopy for chemical selective and ultrasensitive analysis of drugs in aqueous media [1-3]. Hollow-core optical fibers provide a miniaturized sample container for analyte flow and efficient light-guiding. The Raman signals of the antimalarials chloroquine and mefloquine can be strongly enhanced by UV and electronic resonant excitation augmented by fiber enhancement. A fiber adapter assembly was designed for reproducible and quantitative Raman fiber sensing. In doing so the enhanced Raman signals of the pharmaceuticals show excellent linear relationship with sample concentration. This was achieved due to a strong light-analyte interaction in the hollow-core fiber. As the hollow core of the fiber was used as minimized sample container, the performance as optical waveguide was quantitatively analyzed for absorbing samples. Thus it was shown that the ability of fiber enhanced Raman sensing can be significantly improved for trace analysis of pharmaceuticals. Thus a highly improved analytical sensitivity was achieved at minimal sample demand.

Acknowledgment: Funding from the federal state of Thuringia (Forschergruppe Fasersensorik and FaserINFRA) and ESF is highly acknowledged.

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8951-29, Session 6

Raman sensing of drug interference in malaria research

Torsten Frosch, Jürgen Popp, Friedrich-Schiller-Univ. Jena (Germany)

Raman spectroscopy can be exploited for the identification of active agents [2, 3, 5], biological targets [4] and elucidation of drug-target-interactions [1]. This is true because Raman spectroscopy can be applied for a non-invasive, marker-free investigation of molecules in the biological environment, while water does not cause a strong Raman signal. Thus the Raman spectra can be acquired within living cells [4]. By means highly spectrally resolved Raman difference spectra, the weak interaction of heme-targets with antimalarial drugs was elucidated precisely by shifts of the vibrations of the heme macrocycle. These shifts are characteristic for an interaction with a defined stoichiometric ratio of heme to antimalarial active agents. The interpretation of these Raman bands will help in an understanding of the molecular interaction and for the tailored design of new effective active agents.

Acknowledgment: We thank Katja Becker and her colleagues for help with the cell preparation.

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Conference 8952:
Biomedical Applications of Light Scattering IX

Saturday - Monday 1 –3 February 2014

Part of Proceedings of SPIE Vol. 8952 Biomedical Applications of Light Scattering VIII

8952-1, Session 1

Rapid Stokes imaging of turbid media using 2 photoelastic modulators and sequential time gating

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Polarized light imaging is a powerful technique for characterizing turbid media. For various applications, including biomedical assessment, the imaging time should be minimized to avoid motion artefacts. Here, we demonstrate a polarimetric Stokes vector imaging system based on two photoelastic modulators (PEM) and a charged coupled device (CCD) which can retrieve the full Stokes vectors image in millisecond time. PEMs are optical devices which modulate the retardance of light in a sinusoidal fashion with frequencies of tens of kHz [1]. To avoid using lock-in amplifiers, which work well with photodetectors rather than CCDs, a new recovery scheme is proposed. An evolutionary algorithm is used to select four time points at which the system matrix can be inverted to retrieve the full Stokes vector images. However, the PEMs frequencies drift, thus temporal synchronization with the CCD is challenging. To overcome this problem, a field programmable gate array (FPGA) was employed to generate an external trigger for the CCD from the PEMs' controllers' reference frequencies. A CCD with nanosecond gating capability was used to experimentally demonstrate the new approach. From the four CCD intensity images registered in <80 milliseconds, full Stokes images can be recovered.

Our imaging technique is applicable to any turbid media and does not use spatial frequency filtering. We demonstrated the applicability of the system to biomedical applications though imaging Stokes vector of light after interaction with turbid phantoms and ex-vivo tissues. This imaging scheme can be further expanded to Mueller matrix imaging of tissues using 4 PEMs.

1. www.hindsinstruments.com

8952-2, Session 1

Localized differential heterodyne dynamic light scattering system

LiDek Chou, Univ. of California, Irvine (United States) and Chang Gung Univ. (Taiwan); Li-Ping Yu, Chien Chou, Chang Gung Univ. (Taiwan)

In this study, a novel and sensitive localized dual-polarization differential heterodyne dynamic light scattering system for observing in-plane Brownian motion of liquid suspension has been developed. The system uses two highly correlated orthogonally linear polarized laser beams with a slightly difference on temporal frequency. They are incident on sample at different angles that a localized detection volume of Brownian motion in suspension is measured. In this setup, the heterodyne signal is generated by only selecting 180o back scattering photons of two linearly polarized beams. To analyze the heterodyne signal on its spectrum width at beat frequency, the properties of Brownian motion of liquid suspension can be characterized based on the criteria of elastic scattering in dynamic light scattering. In this experiment, the average size of polystyrene and gold nano-particles in water were obtained by best fittings the measured power spectrums with the theory of Brownian motion, while the localization ability of this proposed method was discussed also.

8952-3, Session 1

Biochemical component identification by light scattering techniques in whispering gallery mode optical resonance based sensor

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Experimental data on detection and identification of variety of biochemical agents, such as proteins (albumin, interferon, C reactive protein), microelements (Na+, Ca+), antibiotic of different generations, in both single and multi component solutions under varied in wide range concentration are represented. Analysis has been performed on the light scattering parameters of whispering gallery mode (WGM) optical resonance based sensor with dielectric microspheres from glass and PMMA as sensitive elements fixed by spin - coating techniques in adhesive layer on the surface of substrate or directly on the coupling element. Sensitive layer was integrated into developed fluidic cell with a digital syringe. Light from tuneable laser strict focusing on and scattered by the single microsphere was detected by a CMOS camera. The image was filtered for noise reduction and integrated on two coordinates for evaluation of integrated energy of a measured signal. As the entrance data following signal parameters were used: relative (to a free spectral range) spectral shift of frequency of WGM optical resonance in microsphere and relative efficiency of WGM excitation obtained within a free spectral range which depended on both type and concentration of investigated agents. Multiplexing on parameters and components has been realized using spatial and spectral parameters of scattered by microsphere light with developed data processing. Biochemical component classification and identification of agents under investigation has been performed by network analysis techniques based on probabilistic network and multilayer perceptron. Developed approach is demonstrated to be applicable both for single agent and for multi component biochemical analysis.

8952-4, Session 1

Improving Raman scattering spectroscopy through fluctuation analysis

Christoph Bennenhei, Univ. Oldenburg (Germany); Idir Yahiaténe, Univ. Bielefeld (Germany); Walter Neu, Hochschule Emden-Leer (Germany); Thomas R. Huser, Univ. Bielefeld (Germany); Frank Chuang, NSF Ctr. for Biophotonics Science and Technology (United States)

Raman scattering spectroscopy has been recognized as an effective method to identify and characterize live cells without the use of molecular tags or labels. The technique, however, is often limited by the inherently weak signal and relatively high autofluorescent background and noise in biological samples. Mathematical averaging of repeated spectral measurements is the most common approach to improve accuracy and reduce noise, but mean-value calculations generally do not improve the detection of weak signals that are only slightly above the level of background.

We are investigating alternative methods of acquiring and analyzing Raman spectra to improve the sensitivity and accuracy of this technique. Specifically, by using autocorrelation analysis, we can significantly improve the signal-to-noise ratio, often more effectively than using

mean-value calculations. Also, by modulating the laser excitation power to cause saturation of the autofluorescent signal in a given sample – it becomes possible to identify and subtract fluorescent background from the Raman scattering signal (which does not saturate with respect to the excitation intensity). The combination of these methods to acquire and analyze Raman spectra results in the significant improvement of signal-to-background ratio, as well as enhancement of the resolution of adjacent peaks – without the need of additional measurements or steps to determine the correct background subtraction.

We present the details of this approach and compare the results with those obtained by conventional methods, using computer-simulated Raman spectra. We will also demonstrate how this new approach can be applied to detect viral pathogens in infected human cells.

8952-5, Session 2

MINIMALLY INVASIVE PHOTOPOLYMERIZATION IN INTERVERTEBRAL DISC TISSUE CAVITIES

Andreas Schmocker, Azadeh Khoushabi, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Benjamin Gantenbein-Ritter, Samantha Chan, Univ. Bern (Switzerland); Dominique Pioletti, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Constantin Schizas, Ctr. Hospitalier Univ. Vaudois (Switzerland); Harald M. Bonél, Inselspital, Univ. Bern (Switzerland); Pierre-Etienne Bourban, Jan-Anders E. Månson, Christophe Moser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Photopolymerization of gels is commonly used for a broad range of biomedical applications. As long as the polymer volume is accessible, gels can easily be illuminated and hardened. However, in clinics, especially for minimally invasive surgery, it becomes highly challenging to control photopolymerization. The ratios between polymerization-volume and radiating-surface-area are several orders of magnitude higher than for ex-vivo settings. Furthermore tissue scattering occurs and influences the reaction.

We developed a Monte Carlo model for photopolymerization which takes into account the solid/liquid phase changes, moving solid/liquid-boundaries, refraction on these boundaries and tissue scattering in arbitrarily designable tissue cavities. To validate the model different gels' scattering and absorption coefficients were measured, mechanical properties evaluated using photo-rheology and gels' photopolymerization-induced volume-growth-patterns experimentally verified. Bovine Intervertebral disc-tissue cavities created by enzyme digestion were used to study photopolymerization in-vitro. Magnetic resonance imaging (MRI), tissue histology, cell viability tests and visual inspection tools were employed to investigate photopolymerization outcomes and tissue damage due to photopolymerization.

Monte Carlo model and experimental values were in good agreement. We could show that by adding scattering additives to the liquid monomer the photopolymerized volume is considerably increased. Polymerized volumes could be visualized within real intervertebral-disc-cavities using MRI.

The Monte Carlo model, developed for photopolymerization of a large volume of material through small fibers, provides a tool to tailor both the fiber probe and the scattering/absorption property of the photopolymer for applications such as medical implants or tissue replacements.

8952-6, Session 2

Refractive index determination of single sub micrometer vesicles in suspension using dark-field microscopy

Edwin van der Pol, F. A. W. Coumans, A. N. Böing, A. Sturk, R. Nieuwland, Ton G. van Leeuwen, Academic Medical Ctr., Univ. of Amsterdam (Netherlands)

Background: Cells release vesicles, also called exosomes or microparticles, which are spherical particles containing a phospholipid bilayer. These vesicles are abundantly present in human blood and it is becoming increasingly clear that they contribute to many homeostatic processes, for example coagulation and inflammation. Therefore, vesicles are a potential biomarker for disease. Isolation and detection of vesicles is a major challenge, since vesicles are small (30 nm – 1 ?m) and blood contains similar-sized particles, such as lipoproteins. The refractive index may provide a new label-free parameter to distinguish vesicles from lipoproteins. However, currently no method is available to determine the refractive index of single vesicles in suspension at high throughput.

Method: We have measured the diameter and light scattering of vesicles and beads of known properties by tracking their Brownian motion with dark-field microscopy (NS500, Nanosight Ltd). We analytically described the relation between the diameter, refractive index, and light scattering of beads by Mie theory to determine the refractive index of vesicles from urine and blood.

Results: We obtained a median refractive index of urine vesicles of 1.36, whereas the refractive index distribution of vesicles from blood was much broader and had a median refractive index of 1.49.

Conclusions: Dark-field microscopy can be used to assess the refractive index of single sub micrometer vesicles in suspension. Urine vesicles had a different median refractive index (n=1.36) than particles from plasma (n=1.49). We hypothesize that the relatively high refractive index of plasma particles is due to the presence of lipoproteins.

8952-7, Session 2

Method for rapid multidiameter single-fiber reflectance and fluorescence spectroscopy through a fiber bundle *(Invited Paper)*

Arjen Amelink, Chris L. Hoy, Ute A. Gamm, Henricus JCM Sterenborg, Dominic J. Robinson, Erasmus MC (Netherlands)

We have recently demonstrated a means for quantifying the absorption and scattering properties of biological tissue through multi-diameter single fiber reflectance (MDSFR) spectroscopy. These measurements can be used to correct single fiber fluorescence (SFF) spectra for the influence of optical properties, enabling quantification of intrinsic fluorescence. In our previous work, we have used a series of pinholes to show that selective illumination and light collection using a coherent fiber bundle can simulate a single solid-core optical fiber with variable diameter for the purposes of MDSFR spectroscopy.

In this study, we describe the construction and validation of a clinical MDSFR/SFF spectroscopy system that avoids the limitations encountered with pinholes and free-space optics. During one measurement, the new system acquires reflectance spectra at effective diameters of 200 ?m, 600 ?m, and 1000 ?m, and a fluorescence spectrum at an effective diameter of 1000 ?m. From these spectra, we measure the absolute absorption coefficient, reduced scattering coefficient, phase function parameter gamma, and the intrinsic fluorescence across the measured spectrum. We validate the system using Intralipid and polystyrene sphere-based scattering phantoms, with and without the addition of the absorber Evans Blue. Finally, we demonstrate combined MDSFR/SFF of phantoms with varying concentrations of Intralipid and Fluorescein, wherein the scattering

properties are measured by MDSFR and used to correct the SFF spectrum for accurate quantification of intrinsic fluorescence.

8952-8, Session 3

Subdiffractional length-scale sensitivity of spectroscopic microscopy

Lusik Cherkezyan, Ilker R. Capoglu, Hariharan Subramanian, John E. Chandler, Vadim Backman, Northwestern Univ. (United States)

Optical microscopy techniques are widely used in a variety of biophotonics and medical applications for the study of biological cells and tissues. Nevertheless, the diffraction-limited imaging resolution of optical microscopy restricts the degree of detail it can provide. Emerging “spectroscopic microscopy” (SM) techniques take advantage of the spectral content in the detected light in addition to the imaging benefits of a microscope to overcome the fundamental resolution limit. We have recently demonstrated that spectroscopic analysis of an image obtained by an epi-illumination bright field microscope can reveal the statistics of refractive index distribution beyond the diffraction limit. In particular, we established that the spectral variance of intensity reflectance (Sigma) from a sample with weak refractive index fluctuations quantifies the sample’s internal structure at arbitrarily small subdiffractional scales. Here, we further investigate the size of structures inside weakly-scattering media (such as biological cells) that can be characterized via Sigma. We quantify the length-scale sensitivity of Sigma by measuring its change in response to size-selective removal of specific structures from within a sample. We determine that Sigma is most sensitive to structural alterations at length–scales ranging from 22 to 174nm (1/26 to 1/3 of wavelength). That is, Sigma is highly sensitive to alterations in subdiffractional structures and insensitive to changes in larger structures which, in turn, are naturally resolved in a microscope image. We illustrate the potential of SM and Sigma in detection of early tumorigenic alterations in microscopically-normal appearing cells via an example of nanoscale chromatin reorganization.

8952-9, Session 3

Propagation and scattering of coherent circularly polarized light in turbid random medium

Alexander Doronin, Callum Macdonald, Igor V. Meglinski, Univ. of Otago (New Zealand)

A Monte Carlo computational model for imitating the scattering of coherent linearly, circularly or elliptically polarized laser light and its propagation within turbid biological tissues has been developed. The model had been used extensively to understand the peculiarities of light propagation within tissue-like media. Considering the propagation of circularly polarized and its direction awareness when traveling through turbid media where multiple scattering events occur, light backscattered an odd number of times will correspond to a reversal in helicity, and, thus, contribute to the cross-polarized portion of the detected signal. However, light experiencing an even number of backscattering events contributes to the co-polarized signal, i.e. even number of helicity changes have not changed the handedness of incident polarization of light. The developed model has also been applied to establish optimal parameters of the experimental system developed for cancer detection. The results of modeling and its cross-validation with phantom studies utilizing water solutions of polystyrene micro-spheres of a known size and concentration, as well as the results of measurements for cancer and non-cancer samples in vitro are presented.

8952-10, Session 3

Measuring depth dependent optical properties using diffuse reflectance spectroscopy

Ricky J. Hennessy, Manu Sharma, Mia K. Markey, James W. Tunnell, The Univ. of Texas at Austin (United States)

A two-layer Monte Carlo lookup table-based inverse model is validated with two-layered phantoms across physiologically relevant optical property ranges. Reflectance data for source-detector separations of 370 ?m and 740 ?m were collected from these two-layered phantoms and top layer thickness, reduced scattering coefficient, and the top and bottom layer absorption coefficients were extracted using the inverse model and compared to the known values. The results of the phantom verification show that this method is able to accurately extract top layer thickness and scattering when the top layer thickness ranges from 0 ?m to 550 ?m. In this range, top layer thicknesses were measured with an average error of 10% and the reduced scattering coefficient was measured with an average error of 15%. The accuracy of top and bottom layer absorption coefficient was found to be highly dependent on top layer thickness, which agrees with physical expectation; however, within appropriate thickness ranges, the error for absorption properties varies from 12-25%.

8952-11, Session 3

Comparison among optical blood perfusion measurement modalities: a theoretical study

Renzhe Bi, Jing Dong, Kijoon Lee, Nanyang Technological Univ. (Singapore)

Blood perfusion is of great importance for human health, because it is responsible for transport of oxygen and nutrients to tissue, as well as blood pressure regulation. For blood perfusion measurement, there are several popular optical modalities, laser Doppler flowmetry (LDF), laser speckle contrast analysis (LSCA), diffuse correlation spectroscopy (DCS) and diffuse speckle contrast analysis (DSCA). LDF and LSCA are mainly for superficial tissue blood perfusion measurement, while DCS and DSCA are basically for deep tissue.

People take these optical methods as individual modalities separately for many years. For blood perfusion indicator, LDF uses the shifted frequency, DCS fits the intensity correlation function to certain perfusion model, LSCA and DSCA use statistical analysis of the laser speckle intensity. However all these methods are based on the same principle that the moving particles, mainly red blood cells, can change the autocorrelation function of the back-scattered incident photons. The four optical modalities take different use of the autocorrelation function, and use different indicators to describe blood perfusion dynamics. Thus they can be studied within one same framework.

We compare these theories based on systematically-controlled autocorrelation functions by numerical simulation. They show similar trend as the autocorrelation function decays faster, however the linearity shows different behavior. We also compare different blood perfusion models, such as Brownian motion model and random flow model. This theoretical study gives us a new view to understand these optical modalities, and can provide quantitative comparison among them.

8952-12, Session 3

Multi-wavelength and time-domain diffuse optical tomography data processing by using a material basis and Mellin-Laplace transform

Lionel Hervé, Anne Planat-Chrétien, CEA-LETI-Minatec (France); Laura Di Sieno, Politecnico di Milano (Italy); Michel Berger,

Agathe Puszka, CEA-LETI-Minatec (France); Alberto Dalla Mora, Davide Contini, Gianluca Boso, Politecnico di Milano (Italy); Jean-Marc Dinten, CEA-LETI-Minatec (France)

So as to increase sensitivity in the depth of diffusive media and to separate chromophores with distinct spectral signatures, we developed a method to process time-domain/multispectral diffuse optical acquisitions: 3D Reconstructions of chromophore concentrations are performed with an algorithm based on the use of Mellin-Laplace Transform and material basis. A noise weighted data matching term if optimized by using the conjugated gradients method without expressing the Jacobian matrix of the system. As the algorithm uses reference measurements on a known medium, it does not require measurements or computations of the instrument response function of the system.

Validations are performed in the reflectance geometry on a tissue-mimicking phantom composed of intralipid black ink and a cylindrical blue ink inclusion with a radius of 4 mm located at 15 mm from the probe. The optical tomography setup includes a laser whose picosecond pulses are injected via an optical fiber to the probed diffusive medium and the light collected by a two fibers (located 15 mm apart from the source), is sent to a single-photon avalanche diode (SPAD) operated in the fast-gating mode and connected to a time-correlated single-photon counting board. The source and two detectors scan the surface of the medium so as to provide 30 source-detector couples, 900 time-bins and 5 wavelengths signals. 3D reconstructions performed on the black ink and blue ink materials on a mesh of around 10000 nodes show that we are able to detect, localize and determine the composition of the inclusion and the background.

8952-13, Session 4

Subdiffusion regime reflectance measured by enhanced backscattering to quantify tissue phase function and ultrastructure

Andrew J. Radosevich, Nikhil N. Mutyal, Ji Yi, Elizabeth Horcher, Northwestern Univ. (United States)

Enhanced backscattering (EBS), also known as coherent backscattering, is observed as a 2D angular intensity peak centered in the backscattering direction. By calculating the inverse Fourier transform, this data can easily be converted into the spatially resolved diffuse reflectance profile, P(x,y). While many techniques measure some aspect of P(x,y), EBS specifically targets reflectance at sub-diffusion length-scales (i.e. source-detector separations less than a transport mean free path) with spatial resolution of approximately 10 microns and spatial extent of several millimeters. The advantage of such measurements is the ability to accurately quantify the entire shape of the phase function F as well as any parameters which describe its shape (e.g, anisotropy factor, scattering coefficient, etc). Furthermore, the shape of the refractive index correlation function Bn(r) (through which the spatial distribution of mass is defined) can be analytically derived from the shape of F through application of the Born approximation. Thus, EBS provides measurements of the shape of P(x,y), F, and Bn(r). In this work, we first review the methods used to extract the shape of F and Bn(r) through spectrally resolved EBS measurements. We then apply these methods to measure P(x,y), F, and Bn(r) from tissue biopsies in field carcinogenesis. We find that the changes in these functions' shape are primarily attributable to structures that are 1 order of magnitude smaller than the diffraction limit.

8952-14, Session 4

Comparison of two Monte Carlo models of propagation of coherent polarized light in turbid scattering media

Alexander Doronin, Univ. of Otago (New Zealand); Andrew J. Radosevich, Vadim Backman, Northwestern Univ. (United States); Igor V. Meglinski, Univ. of Otago (New Zealand)

Modeling the propagation of coherent polarized light through a turbid scattering medium using the Monte Carlo method enables better understanding of the peculiarities of image/signal formation in modern optical diagnostic techniques, such as optical coherence tomography (OCT), coherent/enhanced backscattering, laser speckle imaging and diffusing-wave spectroscopy (DWS). Two major ways of modeling the propagation of coherent polarized light in scattering tissue-like media are currently in use. The first approach is tracking transformations of the electric field along ray propagation. Second one is developed in analogy to iterative procedure of the solution of Bethe-Salpeter equation. In this work, we compare these two Monte Carlo approaches by applying them for simulation of coherent backscattering of light. We begin by comparing the accuracy of each technique with the results obtained in experiments. We then discuss the advantages and disadvantages of each technique and their further developments.

8952-15, Session 4

FDTD simulation of an optical absorber based on CPML absorbing boundary condition

Sergio Cantero, Yian Huang, Snow H. Tseng, National Taiwan Univ. (Taiwan)

We propose a method to isolate and selectively remove energy from portions of the scattered field in simulations of biological tissues. This technique will involve placing an absorber in the medium that consists of convolutional perfectly matched layers (CPML). High scattering in most biological tissues limits the applicability of optical imaging techniques, such as optical coherence tomography. Accurate and robust simulations are required due to the complexity of optical wave propagation in these tissues. Recent computational simulations make use of finite-difference time-domain (FDTD) method to exactly solve the scattered electromagnetic field distribution. In this method, CPML absorbing boundary conditions are widely used to terminate the spatial computational grid. However, to overcome the instabilities demonstrated in convex-surfaced PML, we use a Cartesian grid, with a radial dependency of the electrical and magnetic conductivities of the PML impedance-matching condition. Utilizing this approach, the undesired reflected fields are then quantified and compared for different incident light conditions and target sizes. We discuss the performance of the absorber and identify its optimal characteristics.

8952-16, Session 4

A measurement-based analytical approach to the bioluminescence tomography problem

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This work presents a measurement-based analytical approach for the solution of the diffusion equation. We consider a bioluminescent point source with a known location inside the inner region of a two-layered concentric spherical and circular turbid media with different optical properties. The point source is described by the Dirac delta function.

The source strength and the photon density are expressed in terms

of the boundary measurements, the diffusion coefficients, and the associated Bessel functions. Moreover, the methodology is generalized to any finite number of co-linear point sources to obtain the photon density by defining a total transfer matrix which relates the solutions of the innermost region to the solutions of the outermost region. Two main goals of bioluminescence imaging are to determine the distribution and location of an internal bioluminescent source. These goals are generally achieved by means of numerical techniques. In spite of the fact that numerical techniques can be used for complex geometries, they are computationally expensive in practice. On the other hand, analytical approaches can positively affect the computational cost and give more accurate results compared with the numerical approaches. Although these approaches are useful for just some definite geometries, they can generate insight into more complex geometries. For example, a sufficiently long cylindrical medium can be approximated by a 2D polar coordinate system. Therefore, our method offers a different measurement-based analytical point of view to optical imaging techniques like bioluminescence imaging.

8952-17, Session 5

Quantifying the relationship between decorrelation time and blood flow velocity in vivo using sidestream dark field-laser speckle contrast imaging

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Non-invasive imaging and quantification of blood flow in the microcirculation is of critical clinical importance, e.g. to diagnose sepsis, monitor wound healing or assess spatiotemporal development of vasculature surrounding tumors. Laser Speckle Contrast Imaging (LSCI) assesses blood flow in real time but lacks a quantitative relation between speckle contrast (-decorrelation) and flow velocity. Sidestream Darkfield Microscopy (SDF) is able to precisely quantify in vivo flow velocities using frame-to-frame red blood cell tracking (but is limited to low velocities). Here, we integrate both methods to quantify microcirculatory flow through LSC-decorrelation times in the overlapping range of measurable flow velocities. Hereto, we modified the SDF-geometry so that SDF-imaging mode (broad band green light) and SDF-LSCI mode (red laser light) can be consecutively used to image the substrate of interest. We applied SDF-LSCI to flow phantoms and the in vivo microcirculation, allowing the direct comparison between speckle contrast decorrelation times (obtained using a multi-exposure acquisition scheme) and actual flow velocities (obtained using frame-to-frame analysis). Our experiments led to a novel analysis approach based on distinguishing the decorrelation due to blood flow from other additive decorrelation sources in the surrounding tissue. Finally, we quantified the relationship between decorrelation time and absolute flow velocity in vivo and in vitro.

8952-18, Session 5

Optical Properties Assessment for Liquid Phantoms Using Fiber Based Frequency-Modulated Light Scattering Interferometry

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In the present work, fiber based frequency-modulated light scattering interferometry (FMLSI), retrieving the optical properties from the heterodyne interference signal of a Mach-Zehnder interferometer utilizing a frequency modulated coherent light source, is presented. In the interferometer, beat signals are produced due to the time delay between the scattered light and reference light, and the beat frequency is linear proportional to the delay time. In a turbid medium, the scattered light wave with a time-of-flight distribution will produce series of beat signals with corresponding frequencies. Thus, the power spectrum of the detected light intensity is equivalent to the time-of-flight distribution for light that has passed through the turbid media. However, in a liquid turbid medium, apart from the frequency delay due to the light scattering, the Doppler shifts due to the moving particles are also introduced. Thus, the Power spectrum of the detected light intensity would be the combined effect of the Doppler shifts and light scattering, and from which the optical properties could be retrieved based on the diffusion approximation theory. The present FMLSI system, by utilizing multimode fibers to deliver and collect light, is particular convenient for optical properties assessment for many turbid media, and comparison measurements between FMLSI and time-of-flight spectroscopy (TOFS) are also performed with the same detection geometry. The experimental results show the great potential for the present system to be used in many biomedical applications.

8952-19, Session 5

Detecting apoptosis in vivo and ex vivo using spectroscopic OCT and dynamic light scattering

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During apoptosis a series of predictable and dynamic morphological changes occur at the cellular level. Optical coherence tomography (OCT) is sensitive to these changes since both the spectral features of the acquired OCT signal and the speckle intensities in an OCT image are dependent on the size, shape, distribution and optical properties of the scatterers within the imaging sample volume. By measuring spectral parameters such as the integrated backscatter (IB) and the spectral slope (SS) of the backscattered signal, as well as the temporal variation in the speckle intensity (using a dynamic light scattering technique adapted to OCT) we previously detected apoptosis in an in vitro cell death model with acute myeloid leukemia (AML) cells. We have since implemented this AML cell death model in vivo with solid AML tumors grown in the hind leg of 18 SCID mice (6 control and 12 treated). Mice in the treated group were given 150 mg/m2 of intravenous cisplatin. Parameters extracted from OCT data acquired both in vivo and ex vivo (immediately after tumor excision) showed good consistency in the control group with the differences between mice being non significant. Furthermore, good agreement was found between spectral parameters in vivo and ex vivo, demonstrating that blood flow does not significantly affect these parameters in this tumor model. A large variability was observed in the IB, SS and decorrelation time (DT) in the treated groups both at 24 and 48 hours post treatment. This variability was correlated to histological findings showing significant variation in treatment response and tumor microstructure between mice.

8952-20, Session 5

Multimodal optical neural imaging system incorporating laser speckle contrast imaging *(Invited Paper)*

Ofer Levi, Univ. of Toronto (Canada)

We present the development of a multi-modality optical neural imaging system, based on fast coherence reduction techniques applied to Vertical Cavity Surface Emitting Lasers (VCSELs) operating at 680, 795 and 850 nm. We demonstrate a novel system which combines a few techniques including laser speckle contrast imaging (LSCI), near IR fluorescence imaging, and intrinsic optical signal imaging (IOSI), allowing simultaneous wide-field measurement of fluorescence, blood flow velocities and oxygenation changes in the brain. The high brightness, low cost, fast on/off and current switching schemes, and small dimensions of VCSELs make them ideal sources for dynamic neural optical imaging, with fast acquisition rates > 60 frames/sec. Using Multi-exposure speckle analysis in LSCI, we show an improvement in flow velocity estimation over single exposure technique. We have demonstrated that speckle contrast imaging can be an effective label-free technique to monitor the disruption of the blood-brain barrier, through comparing the overall flow in arteries and veins in the effected brain area. Furthermore, we demonstrate monitoring ischemic stroke and seizure activity in a rodent brain, and compare the electrophysiology activity to the hemodynamic response obtain by our system, with high temporal and spatial resolution. Finally, we will present our progress and initial studies with a low-cost CMOS-based portable imaging system as a minimally invasive method for long-term neurological studies in un-anesthetized animals. This system will provide a better understanding of the progression and treatment efficacy of various neurological disorders, in freely behaving animals.

8952-21, Session 6

Scattering orientation imaging and fast tomography via spatial frequency synthesis: towards optical diffusion tensor imaging

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Mapping the three-dimensional structural orientation of tissue is a sought-after feature in medical imaging. Spatial frequency domain imaging (SFDI) is a wide-field, non-contact, near-infrared imaging modality that employs spatially-modulated light to separate scattering from absorption in its measurements. The mean interrogation depth of the modulated light can be controlled by varying its modulation frequency. By using multiple spatial frequencies, it is possible to perform SFDI tomography. However, since multiple spatial frequencies and phases are required to process these datasets, the speed at which we are currently capable of performing tomography is limited. We have developed a new method for processing frames with multiple spatial frequency components, which allows for custom modulation patterns to generate reconstructions with fewer frames of data. SFDI systems typically use digital micromirror devices (DMD) to modulate light. With our new processing technique, we are now able to use mechanical objects instead. As a result, our data acquisition time is no longer limited by the refresh rate of the DMD. In addition to spatial frequency synthesis, we can vary the projection angle of custom patterns to probe tissue orientation. Photons will scatter preferentially along the orientation of tissue structures. We have used this principle to assess the scattering orientation and anisotropy of tissue structures such as collagen. By combining our spatial frequency synthesis and scattering orientation techniques, we will be able to image the orientation of scattering structures in three dimensions, resulting in a new imaging modality called optical diffusion tensor imaging.

8952-22, Session 6

Speckle reduction using wavefront modulation in optical coherence tomography images

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The presence of coherent speckle in optical coherence tomography (OCT) images can obscure identification of small or thin tissue structures. Intensity variation of speckle can blur boundaries between tissue structures with small differences in scattering properties, hindering visualization of small or faintly reflecting morphological features of clinical value. Recently proposed methods for speckle reduction have included digital filters applied during image postprocessing and physical techniques involving compounding of multiple acquisitions of the same area, including frequency and spatial compounding. Although these methods have led to promising results, their drawbacks involve computational intensive postprocessing and extensive system modifications which lead to sacrifice of SNR and resolution as well as lack of real time analysis. We present a method of reducing the effect of speckle in a multifunctional spectral domain OCT system by modifying the wavefront and consequently the speckle pattern using a deformable mirror placed in the sample arm beam path. Our results indicate that by modifying the wavefront between each depth profile acquisition and subsequently incoherently averaging adjacent depth profiles, we can achieve a considerable reduction in speckle contrast. We demonstrate the results of our technique on samples including bovine retinal nerve fiber layer & human skin (intensity), chicken muscle/tendon (polarization), and flowing intralipid solution (flow).

8952-23, Session 6

Biodynamic imaging to predict lymphoma response to therapy

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Lymphoma is one of the most common neoplasms in dogs[1], originating in lymphoid tissues. In the absence of treatment, most dogs with lymphoma diewithin6 weeks.Although doxorubicin is an effective chemotherapeutic agentagainst most caninelymphomas, the duration of doxorubicin response varies dramatically among canine patients, and the treatment is expensive and toxic to some dogs. We have developed a technology called biodynamic imaging (BDI) that uses low-coherence gated digital holography[2] to detect intracellular dynamics and response to treatment through dynamic light scattering. Intracellular motion provides a suite of endogenouscontrastfor the imaging of living tissue. Previously, BDI has been shown to have the ability to perform tissue assessment to track the drug response of in vitro and ex vivo living tissue targets[3]. Here we show that BDI successfully tracks the anti-cancer drug responses of lymphoma biopsies from the canine patients. BDI shows different drug responses (doxorubicin-resistant versus doxorubicin-sensitive) of different dogs for whom the co-response spectrograms are completely different. In clinical practice, chemotherapy treatment protocols are selected empirically, and there are no validated methods for individualizing therapy for specific patients. By comparing the drug response results from BDI with the canine chemotherapeutic responses, we demonstrate that BDI can predict the responsiveness of individual patients' tumors to specific drugs. Therefore, biodynamic imaging has strong potential foroptimizing clinical cancer therapy selection for individual patients.

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[2] D. D. Nolte, R. An, J. Turek, and K. Jeong, "Holographic tissue dynamics spectroscopy," Journal of Biomedical Optics, vol. 16, Aug 2011.

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8952-24, Session 6

Impact of spatial averaging of dynamic speckle on relative blood flow-speed measurement

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Fercher and Briers [1] developed the technique called Laser Speckle Contrast Analysis (LASCA) for visualization of blood flow in the retina; however, its range of application covers different areas of medicine. For long time the LASCA community employed a speckle-to-pixel size ratio equal to unity [1], recently Kirkpatrick et al [2] showed that the speckle size should exceed 2 pixels/speckle to maximize the contrast of the speckle pattern. In this work we show in-vitro experimental data from a study on the effect of the number of pixels per speckle. Our results indicate that speckle contrast increases asymptotically with the number of pixels per speckle, even for ratios that exceed the Nyquist sampling criterion. However, measurements of relative changes in flow speed do not depend on the number of pixels per speckle. Our data also demonstrate that use of the temporal LASCA algorithm, is more accurate at assessing relative changes in blood flow, especially at faster flow speeds, than the spatial LSI algorithm.

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[2] J. Kirkpatrick, D. Duncan, and M. Wells-Gray, "Detrimental effects of speckle-pixel size matching in laser speckle contrast imaging," Opt. Letters 33, 2886-2888 (2008).

8952-25, Session 7

Probing tissue multifractality using multi-resolution analysis for early detection of cancer *(Invited Paper)*

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The inverse power law spectral dependence of the elastic scattering intensity from biological tissue is known to originate from the statistical self-similarity (fractality) of the spatial distribution of tissue refractive index (RI). Recent studies have thus explored quantification of the fractal micro-optical properties, namely, Hurst exponent (H, 0< H<1) and fractal dimension (Df), from tissue light scattering spectra, for their potential applications in precancer detection. The inverse light scattering models developed for this purpose, are usually based on monofractal hypothesis, which assumes that the scaling properties of the RI spatial fluctuations are the same over the entire tissue region probed. Owing to the wide range of dimensions of inhomogeneities and the complex nature of the spatial correlations present between them in tissues, the RI fluctuations in tissue may deviate significantly from monofractal behavior and may exhibit multifractality (multi-scale self-similarity). We have thus investigated this aspect by quantifying the RI fluctuations in tissue using a more general type of statistical multi-resolution analysis, namely, multifractal detrended fluctuation analysis (MFDFA). The differential interference contrast (DIC) images (which provides a direct measure of the spatial distribution of tissue refractive index) obtained from human cervical tissue section (epithelium and connective tissue sections, having different grades of precancers) were analyzed via the MFDFA. The multi-resolution analysis revealed clear evidence of multifractality, yielding its characteristic signatures, presence of long range correlations, non-stationarity in fluctuations and different local scaling behavior. The resulting multifractal trends in tissue RI fluctuations were quantified via the moment (q) dependent generalized Hurst exponent (h(q)), and width of singularity spectrum (??). The analysis yielded intriguing differences in multifractal trends between tissues having different grades of precancers. Specifically, the differences were more prominent in the connective tissue regions of the tissue sections. While the estimates for the generalized Hurst exponent (H = h(q=2)) were found to be considerably lower at higher grades of pre-cancer, the value for ?? were significantly higher. While the former trend indicates that the spatial index fluctuations are more anti-correlated at the higher grades of precancers, the latter points towards stronger multifractality. These differences were attributed to the morphological alterations associated with the micro-architecture (of the fibrous network for the connective tissues), the predominance of index inhomogeneities having smaller spatial dimensions and increased heterogeneity at higher grades of pre-cancer. Note that in Born approximation of light scattering, the light scattering signal (either the wavelength or the angular variation of scattering) can be represented as a Fourier transform power spectrum of the local refractive index fluctuations. Thus, the nature of the spatial fluctuations of RI should also manifest in the Fourier domain as corresponding fluctuations in light scattering spectra. Indeed our initial

light scattering spectroscopic studies from the same tissue samples exhibited similar multifractal trends (as observed in the DIC images), indicating that the tissue multifractal can be quantified from light scattering data as well. The details of the MFDFA analysis on spatial fluctuations of tissue refractive index data, inverse multifractal analysis on tissue light scattering data will be presented and the potential of multifractal parameters for pre-cancer detection will be highlighted.

8952-26, Session 7

Depth-resolved dynamics of aceto-whitening in rabbit cornea studied by 1300-nm optical coherence tomography

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Cervical cancer is the eleventh most common cancer in the UK, especially for women under 35. In developed countries, cervical cancer is diagnosed by performing colposcopy. Contrast is enhanced by spraying dilute acetic acid onto the surface of the tissue. In the past decades, it has been shown that abnormal cervical epithelium turns opaque white upon contact with this weak acid whereas normal epithelium is generally not affected. This mechanism is known as aceto-whitening. However, the exact mechanism of this phenomenon is not fully known.

In this study, OCT using near infrared light was used to quantify depth-resolved kinetics of aceto-whitening in a simple squamous epithelium model: rabbit cornea. We have found that both the epithelium and stroma brighten with approximately the same time course, reaching a peak reflectivity at about 50 seconds. The most significant increase in

reflectivity was seen in the first 20 seconds upon the application of acid, and was measured to be 11dB. This result is compared with phosphate buffered saline solution, which was shown to exhibit no effect.

Lactic acid, an alpha-hydroxy acid, has been reported as a negative control for aceto-whitening. However, our OCT results showed equivalent significant epithelial brightening effect of approximately 8 dB in the first 20 seconds. The key difference with acetic acid is the lack of brightening in the corneal stroma. This could be due to inability to permeate through the basal lamina between corneal epithelium and stroma or lack of interaction with stromal keratocytes. However, the specific reason was not investigated.

Our observations might provide a different perceptive on aceto-whitening in a more clinically approach.

8952-27, Session 7

The study of dynamics of breast cancer oxygenation under neoadjuvant chemotherapy

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Assessment of the oxygen state of breast cancers in the course of neoadjuvant chemotherapy offers the opportunity of understanding the biological changes of tumor tissue that can lead to more individualized treatment. The goal of our pilot study was the detection of oxygenation level of breast cancer using diffuse optical spectroscopy before treatment and three weeks after the first course of chemotherapy and its comparison with tumor pathologic response. Eight patients with locally advanced breast cancer (stage II-IIIA) who underwent neoadjuvant chemotherapy as a standard-of-care treatment were included into the study. Experiments were performed by frequency-domain diffuse optical spectroscopy on the setup with single source-detector pair and parallel plane geometry (Institute of Applied Physics RAS, Russia). The laser source wavelengths are 684 nm, 794 nm, and 850 nm. Concentration of deoxyhemoglobin and oxyhemoglobin, as well as oxygen saturation level were reconstructed. After surgery, macro- and microscopic images of resected tissue specimens were used to categorize the subjects with complete or incomplete responses to therapy. The main predictive criterion allowing evaluating chemotherapy effectiveness appeared to be a trend of tumor oxygenation changes. A complete response (necrosis, the absence of viable cells) was revealed in patients who demonstrated an increase of tumor oxygenation after the first cycle of chemotherapy comparing with its initial level. Incomplete response correlated with a decreased or stable tumor oxygenation. The obtained results suppose that dynamics of breast cancer oxygenation after the first course of neoadjuvant chemotherapy may be a predictive factor of tumor response to treatment.

8952-28, Session 7

Application of angle-resolved low coherence interferometry (a/LCI) to inflammatory bowel disease

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Angle-resolved low coherence interferometry (a/LCI) is an optical biopsy technique used to measure the average size and optical density of cell nuclei in epithelial tissue in order to determine tissue health. The angular distribution of elastically scattered light from cell nuclei and other small scatterers in the target tissue is collected and compared to Mie Theory. a/LCI obtains depth-resolved measurements without the use of exogenous contrast agents, with submicron accuracy. a/LCI has successfully been used to detect the presence of dysplasia in Barrett's Esophagus patients as well as in ex vivo human intestinal and cervical tissue with high sensitivity and specificity.

Here we use a/LCI in an in vivo pilot study in order to assess a/LCI as a means to improve screening of inflammatory bowel disease (IBD). An increased surveillance routine is necessary to search for pre-cancerous conditions in IBD patients, using endoscopy with periodic biopsy, often requiring many biopsies. This regimen is limited as it only examines a small fraction of the colon epithelium.

The a/LCI engine will be adapted for use with a standard colonoscope by a fiber probe that allows a/LCI measurement through the accessory channel. The compatible a/LCI system will be tested in vivo on patients undergoing routine screening for IBD. Each will receive the standard care for monitoring, consisting of 20-50 biopsies along the affected colon. Prior to each biopsy, the tissue will be scanned with a/LCI and the measurements will be compared with pathological evaluation to assess the method accuracy.

8952-29, Session 8

Rapid and label-free identification of individual bacterium with Fourier transform light scattering

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Rapid identification of bacterial species is crucial in medicine and food hygiene. For instance, sepsis, caused by a rapid and severe bacterial infection, results into a deadly whole-body inflammation, which requires the rapid identification of bacterial species and appropriate cures. However, conventional bacterial identification techniques require time-consuming culturing and intensive biochemical treatments, and thus do not match this time requirement. Recent research has seen that several optical methods based on light scattering can be used for assessing bacterial suspension or colonies. However, the identification of bacterial species at the single bacterial cell level has not been fully addressed, mainly due to the limited measurement sensitivity.

Here, we present a rapid and label-free methodology for identifying bacterial species at the individual cell level. Employing quantitative phase imaging (QPI) and Fourier transform light scattering (FTLS) techniques, we demonstrate different bacterial species can be distinguished at the individual cell level. In FTLS, single holographic measurement with QPI provides the far-field scattering map containing structural and compositional information of a bacterium. Four rod-shaped bacterial species were systematically investigated and we demonstrate that the FTLS signals from individual bacterial cells can be used for the identification of bacterial species. Furthermore, we show that the FTLS

signals from individual cells can also be used as an indicator for cell cycle or cell growth. We expect that the QPI-based bacterial studies can potentially be utilized for the various studies in microbiology.

8952-30, Session 8

Time-lapsed study of mitochondrial swelling by angular-domain scattering interferometry and Raman spectroscopy

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We demonstrate a label-free method of simultaneously measuring chemical and structural changes in a single cell over time. Raman spectroscopy obtains chemical information. Angular scattering allows estimates of organelle sizes. Previously, organelle size estimates in single cells were unstable due to the effects of speckle. The small illuminated field causes speckle to affect measurements of single cells more than measurements of many cells or bulk tissue. These speckle effects have been overcome through angular-domain scattering interferometry (ADSI), which records a complex scattered field that can be numerically propagated to any plane. In the image plane, we apply a series of virtual diffusers that reduce the coherence between different intracellular scatterers. Such diffusers are most easily created in silico, necessitating manipulation of the full complex field. By reducing the coherence between scatterers, ADSI is able to reduce the effects of speckle and obtain stable organelle size estimates from single cells. To demonstrate the effectiveness of ADSI on a biological system, we induce mitochondrial swelling and measure the organelle sizes of single cells over the next several hours. Additionally, we acquire Raman spectra from the same cells to examine chemical changes during the swelling process. ADSI and Raman spectroscopy are both well-suited to study a variety of changes in single cells over time. Prospective cellular systems for study by these methods will be discussed.

8952-31, Session 8

Diagnostic features in two-dimensional light scattering patterns of normal and dysplastic cervical cell nuclei

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Dysplastic progression in epithelial tissues is linked to changes in morphology and internal structure of cell nuclei. These changes lead to alterations in nuclear light scattering profiles that can potentially be monitored for diagnostic purposes. Numerical tools allow for simulation of complex nuclear models and are particularly useful for quantifying the optical response of cell nuclei as dysplasia progresses. In this study, we first analyze a set of quantitative histopathologic images from twenty cervical biopsies stained with Feulgen-thionin. Since Feulgen-thionin is stoichiometric for DNA, the images enable us to obtain detailed information on size, shape, and chromatin content of all the segmented nuclei. We use this extensive data set to construct realistic three-dimensional computational models of cervical cell nuclei that are representative of four diagnostic categories, namely normal or negative for dysplasia, mild dysplasia, moderate dysplasia, and severe dysplasia or carcinoma in situ. We then carry out finite-difference time-domain simulations to compute the light scattering response of the constructed models as a function of the polar scattering angle and the azimuthal scattering angle. The results show that these two-dimensional scattering patterns exhibit characteristic intensity ridges that change form with progression of dysplasia; pattern processing leads to extraction of diagnostic features that can be used to distinguish between normal and dysplastic nuclei. Our numerical study also suggests that different angular ranges need to be considered separately to fully exploit the diagnostic potential of two-dimensional light scattering measurements.

8952-32, Session 8

Investigation of the correlation between acetic acid-induced structural changes and backscattering of epithelial cells based on three-dimensional refractive index distributions of living cells

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Acetowhitening, epithelial tissue appearing white after addition of acetic acid, has been used to enhance the contrast for differentiating precancerous or malignant lesions from normal tissue in the cervical and oral mucosa based on higher intensities of backscattered light in abnormal tissue. Previous studies have investigated acetic acid-induced changes in backscattered light of cultured cells, isolated cellular organelles and ex vivo biopsy specimens with light scattering measurements and reflectance confocal microscopy. The increased backscattering caused by acetic acid has been hypothesized to originate from polymerization of cytokeratin in cytoplasm and deacetylation of histones in the nucleus due to a decrease in pH. However, the correlation between acetic acid-induced structural changes and enhanced backscattered light has not been fully elucidated. To address this issue we measured three-dimensional (3D) refractive index (RI) distributions of living cells with recently developed digital holographic microtomography before and after treatment of 0.4% acetic acid solution. Backscattering from local regions such as nucleoli, the nucleus and cytoplasm was investigated by a 3D finite-difference time-domain simulation tool incorporating a focused Gaussian beam for illumination and a confocal pinhole for detection. The addition of acetic acid results in many high RI aggregates in the nucleus, around the nuclear membrane, and throughout the cytoplasm; the level of disorder in the cytoplasmic RI distribution also increases. The increases in high RI aggregates and disorder of the cytoplasmic RI distribution significantly enhance the intensity of backscattered light. The results of investigations of subcellular structures and enhancement factors of backscattering due to acetic acid of epithelial cells present that the nuclei contribute significant high backscattering intensities than those of cytoplasm.

8952-41, Session PSun

A noninvasive diffuse reflectance calibration-free method for absolute determination of exogenous biochemicals concentration in biological tissues

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This paper presents a new method for distant determination of concentration of light absorbing admixtures in turbid media. It is supposed that there is a wavelength region where the absorbent has a narrow absorption peak and medium absorbance (without absorbent) varies smoothly. Main application of the method is diagnostics (photodynamic one, in particular) where the turbid medium is biological tissue, and the absorbent is biochemicals (photosensitizer in PDD). The method uses a probe with 3 optical fibers: one illumination fiber for light delivery from white source to media, and two reading fibers for delivery of

reflected light from media to spectrometer.

Determination of admixture concentration includes two stages, which are performed in one measurement-calculation procedure: evaluation of medium optical parameters at the edge of absorption peak (with method developed by us: A.Lappa, et al, Proc. of SPIE 8579, 857912 (2013)), and calculation of concentration from spectra at the peak.

There are several features in the method:

the value to be determined is absolute concentration of admixtures;

the method needs no calibration measurements on phantoms with given admixture concentrations;

it needs no reference measurements on sample with zero admixture concentration;

it uses a two parametric kinetic model for description of light propagation in medium and no questionable assumptions (diffusion approximation for light propagation, Bragg's rule for determination of mixture absorption coefficient, Mie theory for scattering coefficient and so on);

it uses original algorithms to resolve direct and inverse tasks of radiation transport theory, including Green function approach, the similarity transformation, and a special non-analog correlated technique of the Monte Carlo method.

Tests on tissue phantoms showed good performance of the presented method.

8952-42, Session PSun

A laminar optical tomography system for early cervical cancer diagnosis

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Laminar optical tomography (LOT) is a new mesoscopic functional optical imaging technique which is an extension of a confocal microscope to acquire both the coaxial and off-axis scattered light at the same time. In this paper, a LOT system for the in vivo detection of early cervical cancer is developed. In order to place the target at detection surface accurately and minimize the deviation of high-aperture diffusion light, an automatic focusing detection method with proper aperture diaphragm to mainly receive perpendicular diffused light is proposed. The performance of the system with aperture diaphragm is assessed by solid phantom experiments. The results show that with the method proposed in this paper, the autofocus time are less than 5 s, focusing accuracy reaches 95%. With the results from Monte Carlo simulation as the standard, the measurement results show that the average relative errors of seven different source-detector distances corresponding to 4 source points are 11%?14%?10%?12% respectively, which are lower than the errors of the system without the aperture diaphragm.

8952-43, Session PSun

Modeling and experimental validation of angular radiance and distance-dependent radiance in a turbid medium

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Radiance is sensitive to variations in the tissue optical parameters, such as the absorption coefficient ?a, scattering coefficient ?s, and anisotropy factor g. Therefore, similar to fluence, radiance can be used for tissue characterization. Compared with fluence, radiance has the directional information of light intensity. Taking advantage of the added information, we can determine the spectral parameters using a single optode pair, while typical optical sensors such as fluence measure non-directional light intensity requiring the fiber translation several times, which is difficult to implement clinically. This paper explores a new method to detect the light distribution in intralipid -1% liquid phantom through the measurement of radiance. The approach presents the data in the spectro-distance radiance mapping, spectro-angular radiance mapping and angular-distance radiance mapping respectively. The experimental maps are verified with Monte Carlo simulation and simulated data derived from the analytical solution of the radiative transfer equation. As visual maps provide the information of the light distribution in turbid media, a three-demensional mapping with only a single optode pair will present a way for visual tumor diagnose and light dose calculation during the Photon Dynamic Therapy .

8952-44, Session PSun

An adaptive extended Kalman filter for fluorescence diffuse optical tomography of tumor pharmacokinetics

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According to the morphological differences in the vascularization between healthy and diseased tissues, pharmacokinetic-rate images of fluorophore can provide diagnostic information for tumor differentiation, and especially have the potential for differentiation and staging of tumors. To obtain the pharmacokinetic-rate images, fluorescence diffuse optical tomography method is firstly used to acquire metabolism-related time-course images of the fluorophore concentration, and a parameter-estimation process, such as an extended Kalman filtering (EKF), is then employed based on a kinetic model of the fluorophore metabolism. In this paper, we propose an adaptive EKF framework based on a two-compartment model of plasma and extracelluar-extravascular space, which employs a forgetting factor to emphasize the effect of the current data and to enhance the algorithm robustness to the inappropriate initial values and noise. We use simulate data to evaluate the performance of the proposed methodology. The results suggest that the adaptive EKF can obtain preferable pharmacokinetic-rate images than the conventional EKF with a better performance.

8952-45, Session PSun

3D reconstruction of internal structure of animal body using near-infrared light

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To realize three-dimensional (3D) optical imaging of the internal structure of animal body, we have developed a new technique to reconstruct CT images from two-dimensional (2D) transillumination images.

Efficient light illumination technique was devised to obtain transillumination image through the abdomen of a mouse. The transillumination image is blurred due to the strong scattering in the body tissue. We have developed a scattering suppression technique using a point spread function (PSF) for a fluorescent light source in the body. In this study, we have newly proposed a technique to apply this PSF for a light source to the image of light-absorbing structure.

The effectiveness of the proposed technique was examined in the experiments with a model phantom and a mouse. The mouse was held in a cylindrical holder made of transparent acrylic resin. Light traps were installed in the holder to prevent the surface illumination due to the light-guiding effect of the cylindrical wall. Laser light (Ti:Sapphire, 850 nm

wavelength) was illuminated from one side of the holder and the image was recorded with CMOS camera from another side.

Using the proposed techniques, the scattering effect was efficiently suppressed, and the internal organs such as kidneys were visualized. As a result, we could obtain the 2D image similar to that through low-scattering medium. Using the 2D images obtained in many different orientations, we could reconstruct the 3D image. Through this experimental study, the feasibility of the practical 3D imaging of the internal light-absorbing structure of a small animal was verified.

8952-46, Session PSun

Mapping local anisotropy axis for scattering media using backscattering Mueller matrix imaging

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Backscattering Mueller matrix contains rich information of scattering medium. Mueller matrix imaging techniques can be used to detect the microstructure variations of superficial biological tissues, including the sizes and shapes of cells, the structures in cells, and the densities of the organelles. Meanwhile, many tissues contain anisotropic fibrous micro-structures, such as collagen fibers, elastin fibers, and muscle fibers. Changes of these fibrous structures are potentially good indicators for some pathological variations. In this paper, we propose a quantitative analysis technique based on Mueller matrix for mapping local anisotropy axis of scattering media. By conducting both the experiments on microsphere-silk sample and Monte Carlo simulation based on the sphere-cylinder scattering model (SCSM), we extract anisotropy axis parameters from different backscattering Mueller matrix elements. We find that among all the 16 Mueller matrix elements, the m12 and m13 can be used to map the local anisotropy axis for fibers orientation in a full 2π range. The m22, m23, m32 and m33 can only be used to determine axis for fibers orientation in π range. However, compared to the m12 and m13 elements, the m22, m23, m32 and m33 have better signal to noise ratios, which are suitable for accurate measurements of fibers distributing in a narrow range. Moreover, we testify the possible applications of these parameters extracted from backscattering Mueller matrix for biological tissues. The preliminary experimental results of the chicken heart muscle samples show that, these parameters are capable to map the local orientation for muscle fibers. Since many pathological changes including early stage cancers affect the well aligned structures for tissues, the experimental results indicate that these parameters can be used as potential tools in clinical applications for biomedical diagnosis purposes.

8952-47, Session PSun

Mueller matrix decomposition study on anisotropic medium including cylindrical scatterers and birefringent effect

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Biological tissues consist of scattering particles of different shapes, such as cells, organelles and fibrils etc, and ambient media between the scatterers. In this study, we approximate the complex tissues as a mixture of solid spheres infinite length cylinders and the ambient medium. Using Monte Carlo simulations and Mueller matrix polar decomposition (MMPD) method, we examine in detail the relationship between the parameters of the model and the MMPD. Furthermore, we enrich the model to consider the anisotropic cylinder scatterers with different indices refraction in the axial and radial direction and the situation that when the fibers are not in parallel to the extraordinary axis of birefringence. The effect of the different indices of refraction for the cylinder and the relationship of the total retardance with the intersection angle between the cylinder orientation and the extraordinary axis of birefringence are been studied. The results show that the spherical scatterers and birefringent medium contribute to depolarization and retardance respectively, but the cylindrical scatterers contribute to both. The total depolarization contains contributions from both the spherical and cylindrical scatterers, but the relation is not a simple sum. The total retardance is a linear superposition of the retardance due to the birefringent medium and the aligned cylindrical scatterers when the axis of the cylinder coincide the extraordinary axis of birefringent medium. If the orientation of the aligned cylindrical scatterers does not coincide with the extraordinary axis of the birefringent medium, the total retardance decreased as the intersection angle increased, and the change of total retardance by the variation of the birefringent medium is complex. The anisotropic cylinder scatterers bring a relative small effect to the total retardance compared to birefringence medium. The study will help to reveal the physics insight of the parameters from Mueller matrix polar decomposition and provide more evidences for the feasibility of quantitative characterization of complex tissues.

8952-48, Session PSun

Noninvasive blood flow assessment in diabetic foot ulcer subjects using laser speckle contrast imaging technique

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Measuring microcirculatory tissue blood perfusion is of interest for both clinicians and researchers in a wide range of applications. The measurement could provide essential information of the progress of treatment of certain diseases which causes either an increased or decreased blood flow. Diabetic ulcer associated with alterations in tissue blood flow is the most common cause of non-traumatic lower extremity amputations. A technique which can detect the onset of ulcer and provide essential information on the progress of the treatment of ulcer would be of great help to the clinicians. A non-invasive, non-contact and whole field laser speckle contrast imaging (LSCI) technique has been described in this work which is used to assess the changes in blood flow in diabetic ulcer affected areas of the foot. The blood flow assessment at the wound site can provide critical information on the efficiency and progress of the treatment given to the diabetic ulcer patients. The technique may also potentially full fill a significant need in diabetic foot ulcer screening and management.

8952-33, Session 9

Study of chromatin compaction by inverse spectroscopic optical coherence tomography as one mechanism in colorectal field carcinogenesis *(Invited Paper)*

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Field carcinogenesis (also referred to as field effect, field of injury, etc.) is an initial stage of carcinogenesis, characterized by molecular and ultrastructural alterations in otherwise histologically normal-appearing mucosa. There alterations occur throughout an affected organ site

on which background focal tumors may develop as a result of further stochastic genomic and epigenomic events. Because the field effect is antecedent to carcinogenesis and is present throughout an organ, there has been increasing interest to optically detect field effect as a cancer screening method. Previously, it has been reported that field effect can be detected ex vivo and in vivo in colorectal cancer (CRC). However, these findings lack the morphological understanding (i.e. where does the changes arises). Moreover, since the origins of the changes are unknown, the underlying molecular mechanisms are eluded.

Recently, we used inverse spectroscopic optical coherence tomography (ISOCT) to separate epithelium and extracellular matrixes in CRC field effect and found that both compartment contribute to the optical changes. Here, we hypothesized that chromatin compaction is one of the molecular mechanisms. We applied valproic acid (VPA) on colon cancer cell lines as an inhibitor of histone deacetylases (HDACs), which facilitate the chromatin compaction. As a result, VPA treated cells have decompacted chromatin structures. We took ISOCT measurements on treated cell pellets and found that the changes were reversed to what have been observed in previous ex vivo studies. This suggested that chromatin compaction can be one of the molecular mechanisms in CRC field effect.

8952-34, Session 9

Performance comparison of different metrics for spectroscopic optical coherence tomography

Volker Jaedicke, Semih A?caer, Ruhr-Univ. Bochum (Germany); Francisco E. Robles, Duke Univ. (United States); Nils C. Gerhardt, Ruhr-Univ. Bochum (Germany); Marian Steinert, David Jones, Institute for Experimental Orthopaedics and Biomechanics, Philipps-University (Germany); Hubert Welp, Technische Fachhochschule Georg Agricola zu Bochum (Germany); Martin R. Hofmann, Ruhr-Univ. Bochum (Germany)

When light interacts with a scattering medium, the spectrum of the incident light undergoes changes that are dependent on the size of the scatterers in the medium. Spectroscopic Optical Coherence Tomography (S-OCT) is a method that can be used to ascertain the resulting spatially-dependent, spectral information. In fact, SOCT is sensitive to structures that are below the spatial resolution of the system, making SOCT a promising tool for diagnosing many diseases and biological processes that change tissue structure, like cancer.

The most important signal processing steps for SOCT are the depth-resolved spectral analysis and the calculation of a spectroscopic metric. While the former calculates the spectra from the raw OCT data, the latter analyzes the information content of the processed depth-resolved spectra. We combine the Dual Window spectral analysis with different spectroscopic metrics, which are used as an input to colorize intensity based images. These metrics include the spectral center of mass method, principal component (PCA) and phasor analysis.

To compare the performance of the metrics in a quantitative manner, we use a cluster algorithm to calculate efficiencies for all methods. For this purpose we use phantom samples which contain areas of microspheres of different sizes. Our results demonstrate that PCA and phasor analysis have the highest efficiencies, and can clearly separate these areas.

Finally we will present data from cartilage tissue under static load in vitro. These preliminary results show that SOCT can generate additional contrast in biological tissue in comparison to the pure intensity based images.

8952-35, Session 9

Detection of early ocular disease using two dimensional angle-resolved low coherence interferometry combined with optical coherence tomography

Sanghoon Kim, Stephanie Heflin, Sina Farsiu, Vadim Y. Arshavsky, Adam Wax, Duke Univ. (United States)

Angle-resolved low coherence interferometry (a/LCI) is a light scattering technique that combines the sub- cellular sensitivity of light scattering with the depth resolution of Optical Coherence Tomography (OCT). Using the a/LCI technology, depth dependent cellular structural information such as nuclear size and shape can be determined with sub-wavelength precision and accuracy by analyzing variation in the angular dependence of scattered light. In addition, tissue morphology such as the spacing of the cell nuclei can be accessed via analysis of long range correlation due to coherent scattering. The a/LCI has already shown promising results with regards to detecting neoplasia in vivo in esophageal epithelium of Barrett's esophagus patients. Recently, we have developed 2D a/LCI which allows detection of entire scattering field in two dimensions to improve accuracy. The preliminary data demonstrates that there are changes in the organization of the structural characteristics of photoreceptors in an animal model of retinal degeneration that can be quantitatively evaluated by the 2D a/LCI system. Here, we apply the technology to directly measure the structural changes and characterize them within intact mouse eyes. The OCT scanner is integrated into the 2D a/LCI in order to allow direct comparison between the a/LCI measurements of ex vivo tissue and the OCT images of the same tissue. As a combined modality, quantitative biomarkers that could be used in the future clinical applications to predict the onset and progression of neurodegenerative ocular pathologies can be validated by measurements in a well characterized animal model of progressive retinal degeneration.

8952-36, Session 9

Deep tissue multispectral multiple scattering low coherence interferometry *(Invited Paper)*

Thomas E. Matthews, Adam Wax, Duke Univ. (United States)

We report deep tissue imaging of spectroscopic targets by multispectral, multiple scattering low coherence interferometry (ms2/LCI). These measurements are enabled by a scheme to increase signal to noise based on source modulation and digital lock-in detection of the signal.

Targets were embedded in various scattering samples to demonstrate the features of ms2/LCI. Targets were imaged through 90 scattering mean free paths in a 1-cm thick tissue mimicking phantom. We then acquired images of targets through 5 mm of tissue (chicken breast). Acquired interferograms were processed with the short time Fourier transform method to produce images with spectroscopic contrast. Calculated depth-gated absorption spectra were able to differentiate a variety of chromophores, including the biologically relevant species oxy- and deoxyhemoglobin.

The supercontinuum source we used has two major drawbacks: intensity noise across a variety of time scales and a highly structured, noisy spectrum. This leads to noise which is not easily averaged away and incomplete subtraction of common path artifacts. To overcome these problems, we modulated the sample arm illumination at kilohertz rates with a chopper wheel synchronized to the spectrometer camera. Digital lock-in detection based on the chopper trigger signal was performed on individual sample time-frequency distributions. This removed any slowly varying intensity noise and removed the effects of a shifting illumination spectrum, as well as suppressing features not dependent on the sample arm such as the DC artifact and autocorrelation signals in the reference arm. This method was effective in increasing SNR by more than 6 dB and attenuating artifacts.

8952-37, Session 10

Dynamic light scattering optical coherence tomography imaging of cerebral blood flow and intracellular motility *(Invited Paper)*

Jonghwan Lee, Weicheng Wu, David A. Boas, Harvard Medical School (United States)

We introduce an integration of dynamic light scattering (DLS) and optical coherence tomography (OCT). DLS analyzes fluctuations in light scattered by particles to measure diffusion or flow, and OCT uses coherence gating to collect light only scattered from a small volume. Therefore, the integration, named DLS-OCT, enables high-resolution 3D imaging of heterogeneous diffusion and flow. For this purpose, we derived a DLS theory from the OCT signal, developed a fitting algorithm to estimate dynamic parameters from the 4D field autocorrelation function data, and validated the estimations through numerical simulations and phantom experiments. In consequence, DLS-OCT enabled us to simultaneously image the axial and transverse velocities and the diffusion coefficient with the micrometer-scale resolution.

We applied the technology for imaging of dynamics in the rodent cerebral cortex. The velocity map of DLS-OCT imaging visualized both the axial and transverse velocities of cerebral blood flow (CBF), whereas conventional Doppler OCT generally measures the axial one. In the diffusion map, we observed high-diffusion spots whose locations highly correspond to neuronal cell bodies and whose diffusion coefficient agreed with that of the motion of intracellular organelles (i.e., intracellular motility; IM) reported in vitro. DLS-OCT enabled simultaneous imaging of IM and CBF in vivo by utilizing the fact that the former is diffusive while the latter is translational. As an example application, we used the technology to monitor CBF and IM during a brief ischemic stroke, where we observed an induced persistent reduction in IM despite the recovery of CBF after stroke.

8952-38, Session 10

Diffusion-sensitive imaging of gold nanorods in extracellular matrix with polarization-sensitive OCT

Raghav K. Chhetri, The Univ. of North Carolina at Chapel Hill (United States); Wei-Chen Wu, Joseph B. Tracy, North Carolina State Univ. (United States); Amy L. Oldenburg, The Univ. of North Carolina at Chapel Hill (United States)

The dynamics of nano-sized probes in 3D tissue culture models are of significance in the study of disease progression and disease pathogenesis. In biomedical studies, there is considerable interest in resolving nanoscale diffusion of probes in tissue mimicking extracellular matrix (ECM) models. In this study, we use collagen I and collagen I:Matrigel as ECM models, which recapitulate various functions of tissues. The strong polarization dependent scattering of gold nanorods (GNRs) at the longitudinal plasmon resonant frequency makes them ideal probes for use with polarization-sensitive optical coherence tomography (PS-OCT). Using a custom-designed PS-OCT system, we investigate the use of GNRs coated with a non-adherent polyethylene glycol layer as diffusion probes in collagen I and collagen I:Matrigel ECM models. The rotational and translational diffusion rates of GNRs are inferred from autocorrelations of temporal fluctuations in the backscattered cross- and co- polarized optical fields. We demonstrate the diffusion of GNRs to be sensitive to changes in pore sizes of the ECM by varying the collagen concentration and also by the presence of matrix-remodeling fibroblasts. This highlights their applicability in monitoring ECM-remodeling in longitudinal in vitro studies. Additionally, we demonstrate the utility of PS-OCT in contrasting regions lacking GNRs from cross-polarized images of a spheroid formed by mammary epithelial cells in collagen I:Matrigel ECM. Depth-resolving the autocorrelations in a region across the spheroid is shown to simultaneously reveal the rapid diffusion of GNRs in the interstitial space of the ECM and speckle fluctuations from slower motile activities in the spheroid.

8952-39, Session 10

Light energy enhancement in turbid media

Youngwoon Choi, Timothy R. Hillman, Massachusetts Institute of Technology (United States); Wonjun Choi, Korea Univ. (Korea, Republic of); Niyom Lue, Ramachandra R. Dasari, Peter T. C. So, Massachusetts Institute of Technology (United States); Wonshik Choi, Korea Univ. (Korea, Republic of); Zahid Yaqoob, Massachusetts Institute of Technology (United States)

Multiple light scattering events occurring in a turbid medium attenuate the intensity of propagating waves. This significantly hampers the light delivery into a turbid medium and only limited amount of light energy can be delivered to a desired location. As a result, excessive input light has to be directed into the turbid medium to send enough light at the target depth. Here, we propose a method to efficiently deliver light energy to a desired target depth inside a turbid medium. We measure the time-resolved reflection matrix of a turbid medium using a broadband light source in conjunction with a coherent time-gated detection technique. From the measured time-resolved reflection matrix, we derive an incident wavefront that optimizes the backscattered signal corresponding to a specific arrival time. We experimentally implement the desired incident wave pattern using wavefront shaping and systematically verify that this implementation of the unique wavefront leads to enhanced light delivery to a target depth. Unlike recently demonstrated transmission approaches, the current method characterizes the input-output response of a turbid medium at the same port by measuring its reflection matrix. This configuration removes the requirement of accessing the other side of a turbid medium as in transmission geometries, and is therefore more suitable for biological applications requiring signal acquisition in reflection mode. It is expected that the proposed method will lay a foundation for further technology development or efficient phototherapy and deep-tissue in-vivo imaging.

8952-40, Session 10

Scattering changes during neuronal apoptosis using pathlength multiplexed scattering angle resolved optical coherence tomography

Bingqing Wang, Biwei Yin, Jordan Dwelle, H. Grady Rylander III, Mia K. Markey, Thomas E. Milner, The Univ. of Texas at Austin (United States)

Changes of optical scattering properties in neurons undergoing apoptosis have been observed in previous studies of various neuropathies. For example, RNFL scattering properties in glaucomatous eyes results in decreased RNFL reflectance, which was found to be a sensitive, robust and early diagnostic glaucoma indicator. Observed scattering changes in neurons may be associated with structural changes in mitochondrial networks such as disruption of the natural fusion-fission cycle. Measurement of neuronal scattering changes associated with apoptosis may provide insight into neuronal cytophysiology associated with pathogenesis.

In this study, we present a low resolution pathlength-multiplexed scattering-angle resolved optical coherence tomography (PM-SAR-OCT) instrument (1060?30 nm) which is capable of measuring spatial variation in the angular distribution of backscattered light from biological tissues. PM-SAR-OCT uses pathlength multiplexing to separate incident and backscattered light to/from the sample into discrete angular ranges by placing a glass window with a central clear aperture in the sample path of

a standard swept source OCT system.

To demonstrate the application of PM-SAR-OCT to characterize neuronal scattering properties, five crayfish nerve cords were dissected and imaged using PM-SAR-OCT over a one-hour time period. Time variation of intensity ratios of low-angle and high-angle backscattering from nerve cords during neuronal apoptosis was recorded. PM-SAR-OCT data indicate that in nerve cords undergoing neuronal apoptosis ratio of high to low-angle backscatter changes with time. The results are consistent with previous observations of intensified mitochondrial fission during neuronal apoptosis. Study results suggest application of scattering angle resolved OCT may provide useful diagnostic information for various neuropathies.

Conference 8953: Optical Methods in Developmental Biology II

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8953 Optical Methods in Developmental Biology II

8953-1, Session 1

Confocal imaging of whole vertebrate embryos reveals novel insights into molecular and cellular mechanisms of organ development *(Invited Paper)*

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Confocal microscopy has been an invaluable tool for studying cellular or sub-cellular biological processes. The study of vertebrate embryology is based largely on examination of whole embryos and organs. The application of confocal microscopy to immunostained whole mount embryos, combined with three dimensional image reconstruction technologies, opens new avenues for synthesizing molecular, cellular and anatomical analysis of vertebrate development. Optical cropping of the region of interest enables visualization of structures that are morphologically complex or obscured, and solid surface rendering of fluorescent signal facilitates understanding of three dimensional structures. We have applied these technologies to whole mount immunostained mouse embryos to visualize developmental morphogenesis of the mammalian inner ear and heart. Using molecular markers of neuron development and transgenic reporters of neural crest cell lineage we have examined development of inner ear neurons that originate from the otic vesicle, along with the supporting glial cells that derive from the neural crest. The image analysis reveals a previously unrecognized coordinated spatial organization between migratory neural crest cells and neurons of the cochleovestibular nerve. The images also enable visualization of early cochlear spiral nerve morphogenesis relative to the developing cochlea, demonstrating a heretofore unknown association of neural crest cells with extending peripheral neurite projections. We performed similar analysis of embryonic hearts in mouse and chick, documenting the distribution of adhesion molecules during septation of the outflow tract and remodeling of aortic arches. Surface rendering of lumen space defines the morphology in a manner similar to resin injection casting and micro-CT.

8953-2, Session 1

4D multiplexed functional imaging in deep tissue during embryo development *(Invited Paper)*

Ming Zhao, College of Optical Sciences, The Univ. of Arizona (United States); Xiaoyang Wan, Weibin Zhou, Univ. of Michigan (United States); Leilei L. Peng, College of Optical Sciences, The Univ. of Arizona (United States)

We present a deep tissue multiplexed functional imaging method that probes multiple cellular conditions in live embryos during development. The method uses FRET-based biosensors to sense cellular conditions such as calcium and cAMP concentration levels, and uses fluorescence lifetime quantification to interpret the cellular conditions reported by the FRET biosensors. The method is based on Fourier lifetime excitation-emission matrix (FLEEM) spectroscopy [1] that simultaneously measures fluorescence lifetimes at multiple excitation and emission wavelengths in the form of an excitation-emission matrix within 23 microseconds, allowing measurements on multiple FRET biosensors at the same time in live embryos. Whole embryos are imaged in 3D by combining FLEEM spectroscopy with scanning laser optical projection tomography [2], which collects fluorescence emission from the embryos as 2D projections at multiple angles and reconstructs the three dimensional fluorescence intensities and lifetimes of the fluorophores with isotropic spatial resolution throughout the embryo. In zebrafish embryos transiently expressing cAMP FRET biosensor, an increase in cAMP concentration was observed upon physiological stimulus of foskolin and IBMX. Calcium and cAMP concentration levels during embryonic development were monitored with time lapse 3D imaging of the embryo from 12 hours to 22 hours post fertilization, which showed significant spatial and temporal variation. The method opens the door to multiplexed functional imaging of cellular biochemistries in whole live organisms during development.

[1] M. Zhao and L. Peng, Optics Letters. 35,2910. (2010)

[2] R. A. Lorbeer, et al., Optics Express. 19,5419. (2011)

8953-3, Session 1

Quantifying microvasculature during remodeling of yolk sac in developing mouse embryo

Narendran Sudheendran, Kirill V. Larin, Prathamesh Kulkarni, Univ. of Houston (United States); Mary E. Dickinson, Irina V. Larina, Baylor College of Medicine (United States); Badrinath Roysam, Univ. of Houston (United States)

The cardiovascular system is the first organ to be formed during mammalian embryonic development. Studying remodeling of yolk sac vasculature in mouse embryos can provide insights to the development of cardiovascular system itself. In previous studies, we have demonstrated the advantages of using optical coherence tomography (OCT) for live imaging of mouse embryos at different developmental stages. In this paper we describe a method for speckle variance (SV) analysis of data sets obtained using OCT in order to reconstruct 3D images of yolk sac vasculature in E8.5 and E9.5 mice embryos. The speckle-variance images were then processed using edge-enhancing anisotropic diffusion, followed by block-wise maximal fitting of a parametric active contour (sphere). This provided location, scale and likelihood estimates for each seed point, which is used for discarding the background-seeds. These filtered seeds are used to initialize a priority-based multi-scale genetic algorithm optimized for coverage and consciences. The minimum spanning graph(s) computed thereof defines the centerline and segmentation-mask for the vessel network. Manual and semi-automated editing was optionally used. Finally, a comprehensive summary of vascular descriptors such as vessel length density (VLD), vessel segment density (VSD) and tortuosity were computed. The VSD of E8.5 and E9.5 was determined to be 936±255 and 267±201 per mm3, respectively. The VLD of E8.5 and E9.5 was 108±9 and 53±28 mm/mm3, respectively. Tortuosity of yolk sac vasculature in E8.5 and E9.5 was determined to have a similar value of 1.11. These results suggest that OCT is a promising tool for quantitative analysis of yolk sac vasculature remodeling during normal and pathological embryonic development.

8953-4, Session 1

Optical tissue clearing improves usability of optical coherence tomography (OCT) for high-throughput analysis of the internal structure and 3D morphology of small biological objects such as vertebrate embryos

Lars Thrane, Thomas Martini Jørgensen, Technical Univ. of Denmark (Denmark); Jörg Männer, Georg-August-Univ. of Göttingen (Germany)

The interests of diverse biomedical disciplines, such as anatomy, pathology, or developmental biology, frequently focus on small biological objects (e.g. embryos) whose internal structure and three-dimensional (3D) morphology can be visualized only by use of microscopic techniques. The conventional microscopic analysis of the internal structure or 3D morphology of small biological objects is a time-consuming procedure. It requires several subsequent steps of object preparation, such as micro-dissection, chemical or physical fixation, embedding into an appropriate medium, sectioning with a microtome, mounting of the sections on glass slides, tissue staining, digitalization of sections, as well as computer-based 3D reconstruction. Thus, conventional microscopic techniques are not well suited for rapid morphological analysis of a large number of specimens (high-throughput analysis). Optical coherence tomography (OCT) is a non-invasive imaging technique that facilitates the rapid generation of series of successive optical 2D sections through small biological objects at high resolutions (2-30 microns). Thus, OCT might be an ideal imaging technique for high-throughput analysis of the morphology of small biological objects. However, due to light scattering within semitransparent biological objects, the quality of OCT images drops significantly with increasing penetration depth of the light beam. Here, we show that optical clearing of fixed biological objects (embryonic chick hearts; HH-stages 17-28) with methyl benzoate can significantly reduce the light scattering within biological tissues and, thereby, can improve the quality of OCT images. Thus, combined with optical tissue clearing, OCT can be used for high-throughput analysis of the internal structure and the 3D morphology of vertebrate embryos.

8953-5, Session 1

Enhancing imaging depth by multi-angle imaging of embryonic structures

Narendran Sudheendran, Chen Wu, Univ. of Houston (United States); Irina Larina, Mary Dickinson, Baylor College of Medicine (United States); Kirill V. Larin, Univ. of Houston (United States)

Because of the ease in generating transgenic/gene knock out models and accessibility to early stages of embryogenesis, mouse and rat models have become invaluable to studying the mechanisms that underlie human birth defects. To study precisely how structural birth defects arise, Ultrasound, MRI, microCT, Optical Projection Tomography (OPT), Optical Coherence Tomography (OCT) and histological methods have all been used for imaging mouse/rat embryos. However, of these methods, only OCT enables live, functional imaging with high spatial resolution. However, one of the major limitations of conventional OCT imaging is the light depth penetration, which limits acquisition of structural information from the whole embryo. Here we perform OCT imaging from different sides of the embryos that extend the depth penetration of OCT to permit high-resolution imaging of 3D and 4D volumes.

8953-6, Session 2

Simultaneous real-time quantification of blood flow and vascular growth in the chick embryo using optical coherence tomography *(Invited Paper)*

William J. Kowalski, Nikola C. Teslovich, Carnegie Mellon Univ. (United States); Chia-Yuan Chen, National Taiwan Univ. of Science and Technology (Taiwan); Bradley B. Keller, Univ. of Louisville (United States); Kerem Pekkan, Carnegie Mellon Univ. (United States)

Experimental and clinical data indicate that hemodynamic forces within the embryo provide critical biomechanical cues for cardiovascular morphogenesis, growth, and remodeling and that perturbed flow is a major etiology of congenital heart disease. However, embryonic flow-growth relationships are largely qualitative and poorly defined. In this work, we provide a quantitative analysis of in vivo flow and growth trends in the chick embryo using optical coherence tomography (OCT) to acquire simultaneous velocity and structural data of the right vitelline vein and artery continuously over an eight hour period beginning at stage 16 (hour 54). We obtained 3D vessel volumes (15 ?m lateral, 4.3 ?m axial resolutions, 6 μm slice spacing) at 60 minute intervals, taking a B-scan time series totaling two cardiac cycles at each slice. Embryos were maintained at a constant 37°C and 60% humidity during the entire acquisition period through an in-house built chamber. The 3D vessel lumen geometries were reconstructed manually to assess local growth. Velocity, wall shear stress, and pulse frequency were computed from the central B-scan using red blood cell particle image velocimetry. We performed a regression analysis to form a quantitative mathematical relationship between blood flow and luminal growth in the embryo, demonstrating a correlation between changes in flow and vessel diameter. The use of extended OCT imaging as a non-invasive method for continuous and simultaneous flow and structural data can enhance our understanding of the biomechanical regulation of critical events in morphogenesis. Data acquired will be useful to validate predictive finite-element 3D growth models.

8953-7, Session 2

3D correction of conduction velocity mapping in the early embryonic heart using integrated OCT and OM

Pei Ma, Yves T. Wang, Shi Gu, Michiko Watanabe, Michael W. Jenkins, Andrew M. Rollins, Case Western Reserve Univ. (United States)

Cardiac conduction plays an important role in embryonic heart development. Abnormal conduction patterns and velocities, especially at the early looping stages when the hearts are more susceptible to perturbations, could lead to congenital heart diseases. Optical mapping (OM) is a powerful technique for measuring cardiac electrical activity using voltage-sensitive fluorescent dyes. OM collects information from a 3D surface as a 2D projection map, ignoring the curvature of the heart surface and imaging angles, potentially resulting in significant errors in conduction velocity measurements. Because embryonic hearts are fragile, tiny and spatially convoluted, especially in disease models with looping defects as in models of fetal alcohol syndrome, conduction velocities of looping embryonic hearts are difficult to measure and compare based solely on projected 2D maps. We demonstrate an integrated optical coherence tomography (OCT)/OM imaging system, to calculate conduction velocities in looping embryonic hearts based on surfaces provided by OCT. Image registration ensured accurate alignment of OCT and OM images. A 3D electrical activation map of the looping embryonic hearts is visualized for the first time, minimizing artifacts

caused by unusual heart curves in OM. Standard 2D and 3D-corrected conduction velocity calculations were compared at various regions of the heart and 5-50% corrections were recorded. 3D-corrected conduction velocity calculations can provide greater accuracy and enable direct comparisons between normal and abnormal embryos. This also will allow us to determine absolute conduction velocity, thus allowing the study of channels and gap junctions at the early stage of cardiac development.

8953-8, Session 2

Optical methods for cardiac electrophysiology studies in embryonic hearts

Yves T. Wang, Shi Gu, Case Western Reserve Univ. (United States); Andreas A. Werdich, Brigham and Women's Hospital (United States); Pei Ma, Andrew M. Rollins, Michael W. Jenkins, Case Western Reserve Univ. (United States)

Abnormal electrophysiology in developing hearts can affect development, leading to congenital heart defects. Due to the small size of embryonic hearts, there are significant limitations to the use of traditional electrical techniques to study electrophysiology. Optical techniques – optical mapping for recording and optical pacing for stimulation – offer an alternative.

High resolution optical mapping was obtained under di-4-ANEPPS and cytochalasin D with a microscope and CCD camera providing an 880-1750 µm field of view. A novel signal processing algorithm using cumulative normal distribution fitting enabled more accurate and robust detection of upstroke and repolarization. The algorithm was implemented in MATLAB and validated against simulated data and other methods. Optical pacing was performed using a focused 1465-nm single-mode diode laser with a 12-µm diameter beam waist.

Action potentials with and without optical pacing were recorded in excised 2-7 day quail hearts. The novel algorithm provided accurate and precise results at signal-to-noise ratios greater than 2-3 enabling quantitative measurements of activation times, action potential durations, and conduction velocities. Minor differences were observed between paced and unpaced conditions, likely due to heart rate. Pacing-induced, frequency-dependent conduction block was observed in 2 hearts (2 and 7 day).

Optical methods enable electrophysiological studies in embryonic hearts. While optical mapping is a well-established technique, novel signal processing improves the robustness and accuracy under lower signal-to-noise as found in small embryonic hearts, allowing additional quantitative measurements to be made. Optical pacing facilitates improved maintenance of physiological conditions, enables studies of frequency dependence, and potentially allows generation of arrhythmias.

8953-9, Session 2

Gigavoxel timelaps microsocpy of angiogenic sprouting

Urs Utzinger, Brenda Baggett, The Univ. of Arizona (United States); James B Hoying, University of Louisville (United States); Jeffrey A Weiss, University of Utah (United States)

Ideal observations of in-vitro cultures occur with a field of view large enough to observe relevant events, at resolution high enough to match the scale of the biological system and frequently enough to capture key events. We present a system that uses modest means to visualize angiogenic sprouting on a Giga voxel scale over a period of five days and with two hour time intervals. We use a two-photon single beam laser scanning microscope to observe sprouting microvessels in a collagen hydrogel construct. We incubated in a stage top culture chamber while data is continuously recorded. Data is stored in a low cost storage server and the results of a typical experiment transferred between two institutions at transfer rates comparable to external flash drives. Commonly available image processing software was used to process the raw data and free software tools allowed 3D visualization. Using this process, we have successfully captured the dynamic behavior of vessel sprouting which consists of sprouting, regression and reorientation phases as well as the inosculation of two sprouts. These events represent the key initial steps to form the micro vasculature.

8953-10, Session 3

Early abnormal cardiac function linked to alcohol-induced congenital heart defects *(Invited Paper)*

Ganga H. Karunamuni, Shi Gu, Yong Qiu Doughman, Lindsy M. Peterson, Yves T. Wang, Pei Ma, Katherine Mai, Case Western Reserve Univ. (United States); Kersti K. Linask, Univ. of South Florida (United States); Michael W. Jenkins, Andrew M. Rollins, Michiko Watanabe, Case Western Reserve Univ. (United States)

Over 500,000 American women per year report drinking alcohol during pregnancy, with 1 in 5 who also binge drink. Even low levels of prenatal alcohol exposure can produce birth defects in humans. Epidemiological studies suggest that as high as 54% of live-born children with Fetal Alcohol Syndrome (FAS) present with cardiac anomalies, such as valvuloseptal defects. Currently, most studies focus on signaling pathways affected by ethanol exposure, but pay less attention to the role of altered cardiac function, although changes in hemodynamics can profoundly affect cardiac development. We hypothesized that acute ethanol exposure creates early hemodynamic anomalies that contribute significantly to cardiac structural and functional defects. We employed optical coherence tomography (OCT), a non-destructive imaging modality capable of real-time, micrometer-scale resolution imaging. OCT allowed us to accurately map changes in hemodynamic forces (e.g. regurgitant flow) and the resultant structural abnormalities in the live embryo at very early stages, when the trajectory to heart defects can begin. In our studies, avian embryos exposed to ethanol during gastrulation exhibited alterations in overall embryo body flexure, blood flow, shear stress and cardiac cushion development during heart looping stages. At late stages, ethanol-exposed embryos developed valvular heart defects. Our findings correlated early cardiac dysfunction with late-stage congenital heart defects (CHDs). In addition, we demonstrated that functional analyses using our novel technologies could be used as early and sensitive gauges/predictors of cardiac normalcy and abnormalities. These assays would also enable testing of new therapeutic strategies based on early and accurate diagnosis of birth defects.

8953-11, Session 3

Optical coherence tomography and optical angiography reveal novel embryo heart dynamic outflow tract physiology

Brendan Huang, Constance Weismann, Yale School of Medicine (United States); Stephan Jonas, RWTH Aachen (Germany); Tangji Tong, Michael A. Choma M.D., Yale School of Medicine (United States)

Understanding multiple aspects of physiology is important for elucidating the etiology of disease. The field of cardiology exemplifies this approach, where measurements of multiple physiological parameters including ejection fraction, aortic flow velocity, and aortic pulse wave velocity are routinely made in order to robustly quantify heart disease. One unique aspect of embryonic hearts is that the outflow tract is highly compliant, collapsing during diastole and opening during systole. Drosophila melanogaster troponin-I mutants, known as hdp2, have been previously

been shown to exhibit cardiac dysfunction, including impaired peak systolic wall velocity, and have been used as a model for congenital cardiomyopathy. Here, using a multimodal approach including high-speed angiography and optical coherence tomography (OCT), we have more fully characterized the outflow tract dynamics of hdp2 pre-pupae. Using dye angiography, we show that hdp2 mutants unexpectedly have higher flow velocities in the outflow tract compared to wild type. We also confirmed this velocity finding by estimating aortic pulse wave velocity with OCT. By measuring the maximum diameter of the outflow tract at various lengths along the aorta, we additionally found that hdp2 flies exhibit a decreased maximal outflow tract distension. Taken together, these findings are suggestive of a novel type of embryonic heart defect: a dynamic outflow tract stenosis, where a less compliant vessel leads to a smaller gauge outflow tract and higher ejection velocity.

8953-12, Session 3

Binge consumption of ethanol during pregnancy leads to significant developmental delay of mouse embryonic brain

Narendran Sudheendran, Univ. of Houston (United States); Shameena Bake, Rajesh Miranda, Texas A&M Health Science Ctr. (United States); Kirill V. Larin, Univ. of Houston (United States)

The developing fetal brain is vulnerable to a variety of environmental agents including maternal ethanol consumption. Pre-clinical studies on the development and amelioration of fetal teratology would be significantly facilitated by the application of high resolution imaging technologies like optical coherence tomography (OCT) and high-frequency ultrasound (US). This study investigates the ability of these imaging technologies to measure the effects of maternal ethanol exposure on brain development, ex vivo, in fetal mice. Ethanol-exposed fetuses exhibited a statistically significant, 2-fold increase in average left and right ventricular volumes compared to the ventricular volume of control fetuses. These results indicate that OCT is a useful technology for assessing ventriculomegaly accompanying alcohol-induced developmental delay.

8953-13, Session 3

In vivo 3D imaging of medaka fish using SD-OCT for gender differentiation

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Gender differentiation at the very early stage in some species of fish like puffer fish or koi carp can be of commercial interest. Moreover, in developmental research, there is much interest in the development of gonads in various mutant varieties of Medaka fish because of their close resemblance to vertebrates and the presence of X and Y chromosomes. However, commonly used imaging technologies such as (fluorescent) microscopy can only image the transparent parts or the fluorescent organs of the GFP mutated fish. We propose a spectral-domain optical coherence tomography (SD-OCT) system which is capable of in vivo imaging of the gonads in three dimensions at various developmental stages.

The OCT system has a lateral and axial resolution of approximately 19 µm and 3.5 µm, a center wavelength of 840 nm and a bandwidth of 135 nm (FWHM). Three dimensional volume stacks with dimensions of 7.2 mm x 7.2 mm x 2 mm were acquired at 70 kHz depth scan rate. Results from our real time experiments demonstrate the structural and size differences of gonads in male and female Medaka fish at different points in time during early development. Other organs like the gills, heart, and digestive tract were successfully imaged as well. This demonstrates the potential of SD-OCT as an imaging modality for in vivo studies in Medaka fish.

The system will be further improved for axial resolution by increasing the optical bandwidth and to incorporate an image analysis system to automatically differentiate male and female fish.

8953-14, Session 3

Assessment of imaging parameters correlated with the effects of freezing on embryo development

Livia Zarnescu, Helge Sudkamp, Barry Behr, Thomas M. Baer, Audrey K. Ellerbee, Stanford Univ. (United States)

In the IVF clinic, it is common practice to fertilize several embryos at once, but only transfer a small number of those to a patient to maximize the chance of pregnancy while reducing the chance of multiple births. The remaining embryos are then frozen and stored for possible thawing in the future if the patient wants to try to get pregnant again. However, the freezing and re-thawing process is known to be potentially harmful to embryos and it is difficult to assess from conventional brightfield microscopy whether a given embryo has been damaged by this process. We have developed a microscope that can be used to obtain label-free, noncontact 3D images of live embryos and enable the visualization of structures invisible under the microscopes typically used in the IVF clinic.

Using our microscope, we subjected embryos to varying numbers of freezing and thawing cycles and imaged them in between each cycle. We developed an automated image processing technique to extract a variety of parameters specific to each embryo such as cell shape, nucleus position, and distribution of scattering structures inside the cell. We then allowed the embryos to develop in culture to assess their viability, using blastocyst development as a proxy for viability. We found that embryos damaged by freezing had distinctly different parameters from embryos with normal viability. In the future, this technique will allow clinicians to assess which embryos have been damaged by the freezing and thawing process and avoid transferring those back to the patient.

8953-15, Session 4

Rapid pulsatile flow measurements using Doppler optical coherence tomography

Lindsy M. Peterson, Shi Gu, Michael W. Jenkins, Andrew M. Rollins, Case Western Reserve Univ. (United States)

Blood flow is an important measurement for assessing cardiovascular function. Optical coherence tomography (OCT) is a non-invasive imaging modality with high temporal and spatial resolution that in addition to structural images can provide velocity information through Doppler shifts. However Doppler OCT is only sensitive to motion parallel to the imaging beam. One method to measure the actual flow rates without knowledge of the vessel orientation is to integrate the velocities through an en face cross sectional plane. However, to measure pulsatile flows the en face velocity integration method requires either gated 4-D Doppler OCT data or the region of interest imaged must remain small. To overcome these limitations we have used delay-encoding to modify a scanner to produce two illumination beams with a predetermined angular separation that enables calculation of the velocities perpendicular to the B-scan plane. The velocities can then be integrated over the cross sectional surface to obtain the flow rate from singular B-scan images. The rapid nature of this technique allows for visualization and measurement of pulsatile flow rates over the course of the heartbeat. This technique was validated by imaging a capillary tube phantom over a range of flow rates provided by a syringe pump. The angle independence of the technique was also verified by imaging the capillary tube at various different orientations. Additionally, quail embryo yolk sac vessels were imaged before and after

bifurcations to determine if the combined smaller vessels' flow equaled the original vessel's flow rate.

8953-16, Session 4

Functional analysis of drosophila heart development using optical coherence microscopy

Aneesh Alex, Lehigh Univ. (United States); Airong Li, Massachusetts General Hospital (United States) and Harvard Medical Center (United States); Nicole M. Pirozzi, Lehigh Univ (United States); Fengqiang Li, Lehigh Univ. (United States); Rudolph E Tanzi, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Chao Zhou, Lehigh Univ. (United States)

Drosophila melanogaster heart is an important model system for investigating cardiac development due to its simple tubular organization and similarities to vertebrate heart at early development stages. Identification of regulatory genes involved in Drosophila heart development and their impact on cardiac functions is important. In this study, a high-speed non-invasive optical imaging technique, known as optical coherence microscopy (OCM), was utilized to image the Drosophila heart at different development stages of its lifecycle. Three-dimensional and M-mode images of Drosophila heart was obtained from 29 wild-type flies at 2nd instar, 3rd instar, pupa and adult stages. In addition to heart rate, other functional parameters such as heart tube dimensions, fractional shortening and prevalence of arrhythmia were quantified. Heart rate, being the highest at the 2nd instar stage (~310 beats per minute, bpm), decreased at 3rd instar stage to ~253 bpm and further decreased to ~74 bpm at early pupa stage. The heart stopped beating during mid-pupa stage and resumed to beat by late pupa stage (~161 bpm). The average heart rate in an adult fly was ~299 bpm. In addition to heart rate, other parameters also showed significant changes throughout Drosophila's lifecycle. In order to determine functional roles in heart development of the SOX102F gene, Drosophila ortholog of human SOX5 gene, 22 transgenic flies silenced for SOX102F were also imaged. Morphological and functional changes of the mutant heart at different development stages were compared to that of control flies.

8953-17, Session 4

Real-time off-axis photoacoustic microscopy for dynamic imaging of the zebrafish embryo cardiac cycle

Scott P. Mattison, Ryan T. Maxson, Texas A&M Univ. (United States); Ryan L. Shelton, Univ. of Illinois at Urbana-Champaign (United States); Brian E. Applegate, Texas A&M Univ. (United States)

Photoacoustic microscopy (PAM) combines optical excitation with acoustic detection to provide optically limited absorption detection in the lateral dimension and acoustically limited absorption detection in the axial dimension. Since acoustic signals are one thousand times less attenuated in tissue than optical signals, PAM can provide greater penetration depth than comparable optical techniques. Owing to this greater penetration depth, PAM has potential to be used in a variety of biological applications including developmental studies. Previous uses of PAM for imaging dynamic systems have been hindered by the difficulty of collecting and processing data in real time. We have overcome this limitation by utilizing a field programmable gate array (FPGA) in line with a signal digitizer to perform both data collection and data processing. The FGPA enables the collection of a photoacoustic signal as well as necessary data processing to accurately display morphological data. The result is a PAM system capable of producing continuous real time photoacoustic volumes. Utilizing a 100 kHz q-switched laser, we are able to generate 200 by 200 pixel b-scans at a rate of 500 Hz. Here, we demonstrate this data processing using an off-axis PAM setup for acquiring a four dimensional dataset of the cardiac cycle of a developing zebrafish embryo. This dataset was compiled using postacquisition synchronization of real time photoacoustic b-scans.

8953-18, Session 4

Measurement of strain in early stage chicken embryonic heart in vivo using spectral domain optical coherence tomography

Zhenhe Ma, Fengwen Wang, Northeastern Univ. at Qinhuangdao (China)

Embryonic heart development is a dynamic process involving genetic, mechanical, chemical, and biological factors. During the process, the genes determine the generation of the basic elements and how these basic elements are assembled into a properly functional heart. However, this process is also modulated and influenced by the local biomechanical environment to which cardiac cells are exposed. Usually, stress and strain are employed to quantitatively outline the biomechanical environment. Thus, the ability to measure strain in vivo in embryonic heart is one of the key requirements for understanding the mechanisms of cardiac development. Optical coherence tomography (OCT) is a non-invasive imaging modality with high resolution as well as fast imaging capability suited to study early stage cardiovascular development. We describe a novel method to evaluate the in vivo strain and strain rate of the myocardium in early embryonic stage based on the spectral domain OCT image processing. The myocardium was segmented from the serial OCT images, and then the strain and strain rate were calculated. We evaluate strain and strain rate of different stage chicken embryonic heart outflow tract using the new method. The results demonstrate that OCT can be a useful tool to describe the biomechanical characteristics of the embryonic heart.

8953-19, Session 4

Mouse embryo manipulations with OCT guidance

Monica D. Garcia, Saba H. Syed, Baylor College of Medicine (United States); Andrew J. Coughlin, Rice University (United States); Shang Wang, Univ of Houston (United States); Jennifer L West, Duke University (United States); Kirill V. Larin, Univ of Houston (United States) and Baylor College of Medicine (United States); Irina V. Larina, Baylor College of Medicine (United States)

Optical coherence tomography (OCT) is a useful imaging approach for live embryonic phenotyping analysis of mouse models. The resolution of this imaging modality is sufficient to image individual circulating blood cells and small groups of cells in deep embryonic tissues, which is not currently possible with other approaches. We have previously demonstrated that OCT allows visualization of entire live cultured mouse embryos and Doppler blood flow analysis at early embryonic stages (E7.5-E10) as well as different organ systems including brain, eye and limbs at later stages (E12.5 to the end of gestation) in utero. This work is focused on development of an approach for OCT guided mouse embryo microinjections and micromanipulations to allow targeted delivery of signaling molecules, drugs, and cells with high precision. We performed guided microinjections in the embryonic tissues and demonstrated that the microinjection volume can be controlled on the nanolitter scale. For the injection we have used fluorescent dextran solution as well as gold-silica nanoshell suspension (an OCT contrast agent), which demonstrated the novel potential for the gold-silica nanoshells in the embryonic

research. OCT image guidance in combination with live embryo culture can potentially be used for variety of applications such as guided injections of contrast agents, signaling molecules, pharmacological agents, cell transplantation and extraction, as well as image-guided micro-dissections and other micromanipulations. Our studies also reveal novel potential for gold nanoshells in the embryonic research.

8953-20, Session PSun

Non-contact measurement technique for enzymatic reaction of glucokinase

Juan P. Staforelli, María J. Gallardo, Pablo F. Meza, Sergio N. Torres, Héctor A. Mella, Claudio E. Reyes, Ctr. for Optics and Photonics, Univ. de Concepción (Chile)

A non-contact infrared imaging-based measurement technique is applied to quantify the glucokinase's enzymatic reaction. The method is implemented by a long-wave (8-12 [um]) infrared microbolometer imaging array and a germanium-based infrared optical vision system adjusted to the size of the biological sample. The enzymatic reaction is carried out by the glucokinase enzyme, which is representative of the cell's internal dynamics. Such reactions produce a spontaneous exothermal release of energy detected by the infrared imaging system as a non-contact measurement technique. It is shown by stoichiometry computations and infrared thermal resolution metrics that the infrared imaging system is able to detect the energy release at the [mK] range. This allows to quantify the spontaneity of the enzymatic reaction in a three dimensional (surface and time) single and non-contact on-line measurement. The camera is characterized for disclosing its sensibility, and the fixed pattern noise is compensated by the two point calibration method. On the other hand, the glucokinase enzyme is isolated from Pyrococcus furiosus. Therefore, the experiment is carried out by manual injection with graduated micropipettes using 40 [ul] of glucokinase at the surface of the substrate contained in an eppendorf tube. For recording, the infrared camera is adjusted in-focus at 25.4 [mm] from the superficial level of the substrate. The obtained values of energy release are 139+-22 [mK] at room temperature and 274+-22 [mK] for a bath temperature of 334 [K].

8953-21, Session PSun

Anti-translational research: from the bedside back to the bench for reflectance confocal microscopy

Daniel S. Gareau, The Rockefeller Univ. (United States)

The reflectance confocal microscope has made translational progress in recent decades due to factors such as a nice engineering design and high demand in dermatology. The industrial engine has produced a clinically optimized product, the VivaScope from Calibur ID (formerly Lucid Inc.), which has 0.5 micrometer lateral resolution, 0.75mm field-of-view and most importantly, excellent temporal resolution at ~15 image frames per second. These features that make the VivaScope a valuable tool for imaging cells in living humans, may be overlooked in the basic research arena. This work briefly reviews the basics of high spatiotemporal confocal microscopy then presents preliminary imaging results in various fields: neuroscience where axonal growth cones can be seen in reflectance mode to move rapidly in 3D while forming new configurations and calcium spikes in dendrites (fluorescence mode) that are just beyond the visualization temporal resolution, developmental biology where chicken embryo hearts beat at just under the frame rate and where zebra fish embryos show excellent contrast for the melanocytes overlying the spinal column, and in xenopus embryos where labeling with polystyrene microsphere injections enables cell tracking.

Conference 8954: Nanoscale Imaging, Sensing, and Actuation for Biomedical Applications X

Monday - Thursday 3 –6 February 2014

Part of Proceedings of SPIE Vol. 8954 Nanoscale Imaging, Sensing, and Actuation for Biomedical Applications XI

8954-30, Session PMon

Near-field analysis of CdSe quantum dot conjugated core-shell nanoparticle complexes

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We investigate near-field distribution of CdSe quantum dot conjugated core-shell gold nanoparticle complexes numerically for the application of quantum dots as biosensor. A gold nanoparticle of 30 nm diameter was assumed to be covered with a SiO2 shell, where the thickness of SiO2 varies from 7 to 13 nm. CdSe quantum dots were uniformly located on the Au/SiO2 core-shell nanoparticle surface, while surface density of CdSe quantum dots was changed from 1 to 6 per unit area of 25? nm2 (a circle of 5-nm radius). Three-dimensional finite difference time domain method was utilized for the calculation of near field distribution produced by the core-shell nanoparticle complexes. It was found that regardless of the SiO2 shell thickness and quantum dot surface density, the strongest fields are present in the SiO2 shell. Moreover, electric field intensity becomes stronger along the direction of polarization, as well as weaker with the distance from the quantum dot in the radial direction. In order to examine how nanoparticle cores affect the field and the interaction with quantum dots at periphery, electric field intensities within a distance of 5 nm from the nanoparticle, i.e. from the tip of quantum dots, were circumferentially integrated. The results indicate that the electric field intensity increases with SiO2 shell thickness, especially at high quantum dot surface density. Additionally, the electric field intensity increases more significantly with quantum dot surface density; i.e., electric field intensity increases by approximately 2% with an additional quantum dot per unit area.

8954-31, Session PMon

Imaging mesenchymal stem cells containing single wall nanotube nanoprobes in a 3D scaffold using photo-thermal optical coherence tomography

Valerie Barron, Emma Connolly, Hrebesh M. Subhash, Martin Leahy, National Univ. of Ireland, Galway (Ireland); Niall Rooney, Proxy Biomedical Ltd. (Ireland); Frank Barry, Mary Murphy, National Univ. of Ireland, Galway (Ireland)

Despite the fact, that a range of clinically viable imaging modalities such as magnetic resonance imaging (MRI), computed tomography (CT), photo emission tomography (PET), ultrasound and bioluminescence imaging are being optimised to track cells in vivo, many of these techniques are subject to limitations such as the levels of contrast agent required, toxic affects of radiotracers, photo attenuation of tissue and backscatter. With the advent of nanotechnology, nanoprobes are leading the charge to overcome these limitations. In particular, single wall nanotubes (SWNT) have been shown to be taken up by cells and as such are effective nanoprobes for cell imaging. Consequently, the main aim of this research is to employ MSC containing SWNT nanoprobes to image cell distribution in a 3D scaffold for cartilage repair. To this end, MSC were cultured in the presence of 32?g/ml SWNT in cell culture medium (?MEM, 10% FBS 1% penicillin/streptomycin) for 24h as described previously. Upon confirmation of cell viability, the MSC containing SWNT were encapsulated in hyaluronic acid gels and loaded on polylactic acid polycaprolactone (PLCL) scaffolds. After 28 days in complete chondrogenic medium, with medium changes every 2 days, chondrogenesis was confirmed by the presence of glycosaminoglycan (GAG). Moreover, using photothermal optical coherence tomography (PT-OCT), the cells were seen to be distributed through the scaffold with high resolution. In summary, these data reveal that MSC containing SWNT nanoprobes in combination with PT-OCT offer an exciting opportunity for stem cell tracking in vitro for assessing seeding scaffolds and in vivo for determining biodistribution.

8954-32, Session PMon

Imaigng resolution improvement with plasmonic nanostructures to break an optical diffraction limit

Kyujung Kim, Pusan National Univ. (Korea, Republic of)

A significant improvement of imaging resolution has been desired for a long time in optical imaging fields. The improvement is possible to detect extremely small molecular events in live cell or sub-cellular environments. Many imaging techniques have been investigated ranging from confocal to far-field optical superlens imaging and STED microscopy to break a fundamental optical diffraction limit. I have investigated plasmon based super resolution imaging techniques with nanostructures. With more judicious designs of nanostructures, for example using bowties, nano-pyramids or concentric necklace nanolenses, further resolution enhancements in future researches are expected with rapid development of fabrication techniques.

In this presentation, I introduced plasmon based nanoscale sampling methods to break a diffraction limit with nanostructures in total internal reflection fluorescence (TIRF) microscopy. Near-field distributions of localized fields generated by nanostructures with varied characteristics were simulated using rigorous coupling wave analysis to find optimum design parameters of nanostructures. Various fluorescent molecular targets, such as adenovirus, microtubules and proteins, were used to measure the imaging resolution for a broad application. Significant resolution improvements using aperiodic random nanoislands patterns and periodic nanohole structures were achieved down to 70 nm imaging resolution without fundamental deviation from a conventional TIRF microscope. I compared those performances to an extraordinary transmission based super resolution imaging technique using linear nanoaperture arrays, and a merged plasmon based super resolution imaging platform was explored to analyze molecular events with nanoscale eventually.

8954-33, Session PMon

Au/Cu2-xSe nanoparticles with tailored localized surface plasmon resonance as contrast agents for in vivo photoacoustic imaging

Changho Lee, Kyungpook National Univ. (Korea, Republic of); Xin Liu, Wing-Cheung Law, The State Univ. of New York at Buffalo (United States); Mansik Jeon, Chulhong Kim, Pohang Univ. of Science and Technology (POSTECH) (Korea, Republic of); Mark T. Schihart, Paras N. Prasad, The State Univ. of New York at Buffalo (United States)

A new type of heterogeneous nanomaterial, Au/Cu2-xSe heterodimer

nanoparticles (NPs) composed of a heavily-doped semiconductor domain (Cu2-xSe) and a metal domain (Au), which exhibit a broad localized surface plasmon resonance (LSPR) across visible and near infrared (NIR) wavelengths, arising from interactions between the two nanocrystal domains, and their application as a sensitive contrast agent for photoacosutic imaging are reported. The Au-Cu2-xSe NPs exhibit a broad optical absorption that is nearly flat across the near infrared (NIR) spectral region (750-1150nm), along with a small shoulder at 566 nm that originates from the Au NP. This spectrum is significantly different from that of the Au NPs and from that of our previously-reported Cu¬2-xSe NPs. The broad LSPR absorbance enables both dark-field optical imaging and PA imaging with different light sources. The clinical relevance of these new PA contrast agents was demonstrated through deep tissue visualization of a SLN in a rat. Imaging through layers of chicken breast tissue at total imaging depths needed for human SLN imaging was achieved. Further, the kinetics of these NCs in the rat circulatory system were monitored in vivo. A widely available and relatively low cost Nd:YAG laser source (1064 nm) was used for all PA imaging experiments, which is an additional benefit for easy commercialization and clinical translation.

8954-34, Session PMon

Nanoparticles characterization using HDR-NTA image analysis

Raul E. Cachau, Frederick National Lab. for Cancer Research (United States); Bradford C. Braden, Bowie State Univ. (United States); Jack R. Collins, Frederick National Lab. for Cancer Research (United States); Jose R. Casas-Finet, MedImmune LLC (United States)

Nanoparticle Tracking Analysis (NTA) is a powerful technique for the rapid characterization of polydisperse nanomaterials. NTA platforms rely on a laser illumination system coupled with an optical magnification path allowing the rapid and accurate tracking of single particle positions in liquid suspensions. The particle tracking information is then used to estimate the particle hydrodynamic radii and other properties or interest. High Dynamic Range (HDR) photography is a relatively inexpensive way to obtain higher contrast imagery in settings where high contrast information may be lost resulting from over/under exposure or data acquisition time constrains. In this presentation, we will discuss the use of a NTA platform modified to accept up to 12 virtual cameras. Among the advantages of using multiple camera settings in NTA are the increase in total tracking time per particle, and the ability to collect non-saturated images of bright spots. The increased tracking time can be used to improved statistics. The enhanced dynamic range can be used to quantify the particle flickering, which may offer new ways to characterize particle shape fingerprints using NTA platforms. An additional advantage of HDR-NTA approaches is the increased accuracy in the evaluation of particle concentrations and the ability to extend the method to larger particles or aggregates.

8954-35, Session PMon

Soft nanomaterials characterization using low-voltage-high-contrast electron microscopy and advanced image reconstruction techniques

Raul E. Cachau, Frederick National Lab. for Cancer Research (United States); Bradford C. Braden, Bowie State Univ. (United States); Jack R. Collins, Igor Topol, Frederick National Lab. for Cancer Research (United States); Jose Ramon Casas-Finet, MedImmune LLC (United States)

Polymer based nanoparticles are a promising platform for biomedical applications. The characterization of these materials by transmission electron microscopy (TEM) present a number of challenges. Low Voltage Electron Microscopes (LVEM5) instruments operate at an uncommon low voltage (5KeV) resulting in a great increase in contrast. This makes the LVEM5 particularly suitable for the characterization of soft materials (polymers, cellulosic materials, unstained biomolecular aggregates etc.) In this first work we will explore the application of the LVEM5 to the characterization of dendrimers. Dendrimers are characteristically narrowly polydisperse, polymorphous, adaptable platforms ideally suited for the development of engineered nanoxenobiotics. The narrow polydispersion and polymorphisms of dendrimer samples are however, a great challenge to analytical methods that rely on averaged measurements. Images of unstained dendrimers directly deposited on Formvar films were first described by Drummy (Drummy et. al., Ultramicroscopy 99 (2004) 247–256) using an LVEM5. In this work we will discuss the characteristics of the images of dendrimers obtained using an LVEM5 microscope and the especial requirements they impose for their proper processing, and present the first 3D reconstruction of a dendrimer obtained using a single projection reconstruction technique and a structure docking procedure.

8954-1, Session 1

Enhanced coherent anti-Stokes Raman scattering imaging using silica mircospheres

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Coherent anti-Stokes Raman scattering (CARS) microscopy is a powerful imaging technique that can provides chemical imaging information of living cells and organisms based on molecular vibrational spectroscopy. However, its contrast is not enough for monitoring molecules present in low concentrations. One way to achieve higher contrast is to employ silica microspheres to enhance CARS signals. In the study, one layer optically transparent silica microspheres with 3~7 ?m diameter were placed on testing object (for example, polymer grating consisting of 400-nm-wide spaced 400 nm apart) by self-assembly. The narrow-band 800 nm femtosecond pump laser and broadband Stokes laser were focused on the objects by a water-immersed optical lens (NA=1.05, Olympus) in a laser scanning microscopy and a 650/13 nm bandpass filter was used in imaging of CH vibrational signals from forwards direction. The best enhance factor ?2.5 by using 6.02 ?m microspheres was achieved. A simulation based on FDTD was also done to support the experimental results.

8954-2, Session 1

Engineering fluorescence lifetime with fano-resonant plasmonic system

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Over the last five years Fano resonances in plasmonic systems have emerged as a vivid field of research. Fano resonances exhibit very narrow asymmetric spectral features, which are created from the interaction between bright (radiative) and dark (non-radiative) modes supported by a plasmonic nanostructure. Consequently, Fano-resonant plasmonic systems have the potential to produce strongly modified local density of states, depending whether bright or dark modes are excited.

These variations of the density of states can be probed by measuring the fluorescence lifetime of molecules deposited on the plasmonic nanostructure. In this work, we use dolmen-shaped nanostructures made of gold with dimensions in the 100nm range as Fano-resonant nanostructure and measure the fluorescence lifetime for different dye molecules. These dolmen structures can be tuned in a way such that the Fano resonance is positioned at a specific wavelength. The fluorescence lifetime is measured on a series of different structures as a function of the spectral location of the Fano resonance. These results indicate dramatic variations of the lifetime depending on the tuning of the Fano resonance. They are supported by numerical calculations of the radiative and non-radiative decay times at the vicinity of the structure for different Fano configurations. We further explore different coupling regimes between the molecule and the nanostructure, by tuning their spectral overlap. Fano-resonant plasmonic systems appear to be extremely versatile for lifetime engineering and this work paves the way for controlling decay rates by a combination of bright and dark modes in plasmonic systems.

8954-3, Session 1

Two dimensional multispectral imager based on tiled arrangement of metallic nanohole arrays

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Multispectral imaging systems provide valuable spectral information for materials in various states, which cannot be detected by eye. The spectral signatures from absorption, reflection, and transmission have been utilized in a wide variety of applications ranging from remote sensing to cancer detection. However, most multispectral imaging systems are based on spectral or spatial scanning and generally not capable of acquiring full field images of fast-moving objects in a scene.

To avoid spectral or spatial scanning, we designed and prototyped a two dimensional multispectral imager based on a tiled arrangement of nanohole arrays (NHAs). The tiled arrangement enabled unmixing of light at each spatial pixel into multiple spectral bands in the near infrared regime. Prior to fabrication of the imaging device, we performed an analysis on the measured optical transmission of a series of NHAs with differing optical properties and selected 4 NHAs with the least amount of inter-array correlation. Using a linear unmixing algorithm, the transmission due to the illumination from a source with controlled spectral content was successfully unmixed in the component spectral bands using the imager. We then performed near video rate multispectral transmission imaging of an optical contrast agent flowing within a tube and determined that the unmixed images were consistent with the absorption spectrum of the contrast agent. Based on the findings, full field near video rate multispectral imaging is possible with a NHA-based device and could potentially offer a way to obtain multispectral images of fast-moving objects in a scene.

8954-4, Session 1

Nanoscale soft x-ray absorption and phase contrast imaging *(Invited Paper)*

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We demonstrate nanoscale resolution imaging of nanostructures and biological samples using aerial soft x-ray laser microscopes that combine coherent illumination from a compact 46.9 nm wavelength laser with Fresnel zone plate optics. We also show the implementation of image plane holography using these microscopes from which two dimensional absorption and phase maps of the object are extracted.

8954-5, Session 2

Plasmonic crystal based solid substrate for biomedical application of SERS

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Surface Enhanced Raman Spectroscopy is a powerful analytical technique that combines the excellent chemical specificity of Raman spectroscopy with the good sensitivity provided by the enhancement of the signal observed when a molecule is located on (or very close to) the surface of nanostructured metallic materials.

Nanohole array based solid SERS substrates are a promising materials that provide a regularly distributed, high dense, array of hot spots with tunable properties. Moreover, they offer the facile phase separation advantage and the stability of the heterogeneous reaction systems. In these structures, enhanced electrical field is generated when incident light excites an active plasmonic mode of nanoholes, which can be exploited for several plasmonic applications.

The availability of cheap, reliable and easy to use SERS substrates would pave the road to the development of bio-analytical tests that can be used in clinical practice. SERS based analysis in the biomedical field is expected to provide not only higher sensitivity (Graham, 2010) and specificity, but also the simultaneous and markedly improved detection of several target at the same time with higher speed compared to the conventional analytical methods (Kirschner et al, 2001). Several studies forecast the potential of Raman-SERS to yield innovative biotechnological applications in the medical field (Nguyen et al, 2010; Yuen et al, 2010; Sanchez-Cortes et al, 2013).

We present here the SERS activity of 2-D plasmonic crystals deposited by the combination of soft-lithography and plasma deposition techniques on transparent substrates. The transparent support material allowed SERS detection from support side opening the possibility to use these substrates combined with microfluidic devices.

In order to demonstrate the potential for bioanalytical applications, the SERS active gold surface was used to detect the oxidation products of apomorphine, a well-known drug molecule used in Parkinson's disease which has been demonstrated being difficult to study with traditional HPLC based approaches.

8954-6, Session 2

Highly-sensitive measurement of single DNA translocation through an ultraviolet light spot on silicon nanopore

Hirohito Yamazaki, Shinji Kimura, Mutsumi Tsukahara, Keiko Esashika, Toshiharu Saiki, Keio Univ. (Japan); Toshiharu Saiki, Keio University (Japan)

Nanopore-based sensing is an attractive candidate for developing single-molecule DNA sequencing technology. Recently, an optical detection

with massively parallel nanopore array has been demonstrated. Although this method is a promising approach to develop high thorough-put measurement, the approach requires the observation at low-background condition. In this paper, we propose a new optical method for nanopore DNA sequencing with high resolution and high signal-to-noise ratio. We use ultraviolet light for the excitation of a fluorescent probe and a nanopore in a silicon membrane. Because silicon has the large refractive index and extinction coefficient at ultraviolet wavelengths, light transmission thorough membrane is negligible. This contributes to low background measurement of fluorescence from fluorophore-labeled DNA strands. In addition, the z-polarization component of the electric field is expected to generate a large electric field gradient at the nanopore exit due to the boundary condition at the silicon surface. Our numerical electromagnetic simulation revealed that the z-component electric field is dominant compared to the x-component electric filed. The intensity of z-component electric field significantly reduced inside the membrane. For this fact, a considerably large gradient of electric field is anticipated at the exit of nanopore. The electric field rises steeply in 2 nm, when ultraviolet light of 375nm wavelength is focused on 10nm thick silicon membrane with 7nm diameter nanopore. This resolution is sufficient for the sequencing of designed DNA polymer. Finally, we demonstrated optical detection of single DNA translocation event with high signal-to-noise ratio under applied voltage.

8954-7, Session 2

Total internal reflection fluorescence polarization imaging of red blood cells

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The biophysical properties of red blood cells (RBCs) are crucial for a wide variety of life processes, from cell transport in microcapillary blood vessels to cancerogenesis as well as ageing, when blood vessels become stiff. Significant changes in elasticity of RBCs are known to occur under influence of number of diseases such as malaria, cancer, and diabetes. Since the pathophysiology of progression of these diseases is reflected in the mechanical and material properties of the RBCs, we hypothesize change in RBC membrane fluidity occurs with progression of these diseases. Here, we report development of total internal reflection (TIR) fluorescence polarization microscopy and its use for measurement of RBC membrane properties using fluorescent membrane probe (DiOC6). The system utilized a dualview setup to split the two orthogonally polarized TIR-fluorescence images projected on to a sCMOS camera. This allowed high-speed (100fps) imaging of fluorescence polarization of RBC membrane. Use of fluorescence polarization imaging for evaluation of physiological state of RBC will be presented.

8954-8, Session 2

Intra- and extracellular plasmon-enhanced fluorescent Ag@SiO2 nanoparticles ionic sensor

Jeremie Asselin, Carl Roy, Denis Boudreau, Younes Messaddeq, Univ. Laval (Canada)

Intra- and extracellular detection of various ions and molecules is essential for a better understanding of different cell functions. However, even if many measurement methods are already available, most of them are not easily adaptable to give accurate results in situ without interfering with the medium. Therefore, we have developed and optimized a fluorescent architecture based on metal-enhanced fluorescence (MEF) for ionic sensing applications. Our fluorescent Ag@SiO2-FITC core-shell system was thoughtfully characterized for precise pH-sensing and analyses in a physiological range of concentrations. Furthermore, detection of other species in solution was also possible with minute system modifications, resulting in similar sensitivity. Applications for such analytical platforms are numerous in life sciences, going from in vivo measurements in critical situations to in vitro laboratory studies.

8954-9, Session 3

Ultrafast subnanometric spatial accuracy of a fleeting quantum probe interaction with a biomolecule: innovating concept for spatio-temporal radiation biomedicine *(Keynote Presentation)*

Yann A. Gauduel, Victor Malka, Ecole Nationale Supérieure de Techniques Avancée - Ecole Polytechnique (France)

During cancer radiotherapy protocols, the early profile of energy deposition is decisive for the prediction and control of radiationi-induced biomolecular and sub-cellular damage. A major challenge of spatio-temporal radiation biomedicine, a newly emerging interdisciplinary domain, concerns the complete understanding of biophysical events triggered by an initial energy deposition inside confined ionization clusters (tracks) and evolving over several orders of magnitude, typically from femtosecond (1 fs = 10-15 s) and sub-nanometer scales. The innovating advent of femtosecond laser sources providing ultra-short photon beam and relativistic electron bunches, in the eV and MeV domain respectively, open exciting opportunities for a real-time imaging of radiation-induced biomolecular alterations in nanoscopic tracks. Using very short-lived quantum probe (2p-like excited electron) and high-time resolved laser spectroscopic methods in the near IR and the temporal window 500 – 5000 fs, we demonstrate that short-range coherent interactions between the quantum probe and a small biosensor of 20 atoms (disulfide molecule) are characterized by an effective reaction radius of 9.6 ± 0.2 angströms. For the first time, femtobioradical investigations performed with aqueous environments and biomimetic organized assemblies give correlated information on spatial and temporal biomolecular damages triggered by a very short lived quantum scalpel whose the gyration radius is around 6 angströms. This innovating approach would be applied to more complex biological architectures such as nucleosomes, healthy and tumour cells. In the framework of high-quality ultra-short penetrating radiation beams devoted to pulsed radiotherapy of cancers, this concept would foreshadow the development of real-time nanobiodosimetry combined to highly-selective targeted pro-drug activation.

8954-10, Session 3

Plasmonic nanoparticle-enhanced lensfree holographic cytometry

Qingshan Wei, Euan McLeod, Univ. of California, Los Angeles (United States) and California NanoSystems Institute (CNSI) (United States); Hangfei Qi, Zhe Wan, Univ. of California, Los Angeles (United States); Ren Sun, Aydogan Ozcan, Univ. of California, Los Angeles (United States) and California NanoSystems Institute (CNSI) (United States)

Lensfree computational on-chip microscopy has been an immerging biomedical imaging modality due to its large field of view (FOV), compact optical design, and cost effectiveness. In particular, partially coherent lensfree in-line holography is well suited for rapid cell imaging and automated counting on a chip given the significantly enhanced depth-of-field (e.g. ~1-5 mm) over other lens-based imaging modalities. Here, we introduce a novel on-chip cytometer that uses plasmonic nanoparticles (NPs) to enhance cell-specific contrast in lensfree holographic imaging. Using antibody-conjugated metal nanoparticles as targeting probes, the

optical absorption and scattering spectra of labeled cells are modulated as a function of wavelength and are probed by multi-spectral lensfree holographic microscopy. To test the proof of concept of this imaging cytometry platform, using machine learning algorithms, gold-NP labeled CD4+ T cells were imaged, reconstructed, and differentiated from unlabeled CD4 cells with an average classification accuracy of >93%. Multiplexed detection of T cell subtypes such as CD4+ and CD8+ cells was also achieved with more than 95% accuracy via labeling of cells with gold and silver NPs, respectively. Finally, we monitored the NP labeling density on individual T cells and revealed a saturation surface density of gold NPs on CD4+ cells with 150 NPs/um2. This lensfree holographic on-chip cytometer, with a rather large FOV and a long depth-of-field could be quite useful for high-throughput imaging cytometry applications/needs even in resource-limited settings.

8954-11, Session 3

Photothermal optical coherence tomography for depth-resolved imaging of mesenchymal stem cells via single wall carbon nanotubes

Hrebesh M. Subhash, Emma Connolly, Mary Murphy, Valerie Barron, Martin Leahy, National Univ. of Ireland, Galway (Ireland)

The progress in stem cell research over the past decade holds promise and potential to address many unmet clinical therapeutic needs. Tracking stem cell with modern imaging modalities are critically needed for optimizing stem cell therapy, which offers insight into various underlying biological processes such as cell migration, engraftment, homing, differentiation, and functions etc. In this study we report the feasibility of photothermal optical coherence tomography (PT-OCT) to image human mesenchymal stem cells (hMSCs) labeled with single-walled carbon nanotubes (SWNTs) for in vitro cell tracking in three dimensional scaffolds. PT-OCT is a functional extension of conventional OCT with extended capability of localized detection of absorbing targets from scattering background to provide depth-resolved molecular contrast imaging. A 91 kHz line rate, spectral domain PT-OCT system at 1310nm was developed to detect the photothermal signal generated by 800nm excitation laser. In general, MSCs do not have obvious optical absorption properties and cannot be directly visualized using PT-OCT imaging. However, the optical absorption properties of hMSCs can me modified by labeling with SWNTs. Using this approach, MSC were labeled with SWNT and the cell distribution imaged in a 3D polymer scaffold using PT-OCT.

8954-12, Session 3

Photo-switchable quantum dots based on reversible FRET

Qirui Fan, The Ohio State Univ. (United States); Gauri Nabar, Carl Miller, Carlos Castro, The Ohio State University (United States); Jessica Winter, The Ohio State Univ. (United States)

Super-resolution fluorescence microscopy is anticipated to be a powerful tool in observing biological structures and processes smaller than the diffraction limit of light microscopy (~200nm). Yet, many super-resolution techniques (STORM/PALM, STED) employ photo-switchable fluorescent probes (i.e., dyes and fluorescent proteins) that are limited in brightness and stability, reducing potential image resolution. Here, we describe photo-switchable quantum dots (QDs) with enhanced brightness and stability, and excellent optical properties, including narrow emission spectra and broad excitation spectra compared to fluorescent dyes. These QDs are composed of one green QD, one gold nanoparticle (AuNP), and complimentary single stranded DNA (ssDNA) modified with photo-sensitive azobenzene groups bound to each of the particles. Because of the azobenzene photosensitive property, the ssDNA strands hybridize when excited with visible light, yielding a QD-AuNP conjugate in which QD fluorescence is quenched through Förster resonance energy transfer (FRET); and dehybridize under visible light, yielding separate QDs and AuNPs that are free to diffuse from each other. Because FRET is strongly distance dependent (i.e., ? 1/r6, in this case a few nanometers), QD fluorescence is restored. These QD “light-switches” exhibit a fluorescence intensity ratio between the bright state and dark state of 150%, with reversible switching. Compared with other light-sensitive QDs, this design displays stochastic on/off switching, which is a key requirement for single-molecule localization-based super-resolution imaging techniques (i.e., STOMR/PALM).

8954-13, Session 3

Live cell imaging based on surface plasmon-enhanced fluorescence microscopy using random nanostructures

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Recently many imaging techniques have been developed to achieve imaging resolution below diffraction limit for cell microscopy and molecular analysis and to improve detection of biomolecular events in cellular environments. Of particular interest here are surface plasmon (SP) enhanced imaging techniques in which emission of fluorescence in the evanescent field is localized at surface relief metal nanostructures for high resolution microscopy. In this study, we explore demonstration of localized SP enhanced microscopy for live cell imaging based on nanoislands of random spatial distribution. An important advantage of the technique is the ease of fabrication steps that take to produce random island patterns without need of lithographic techniques. Nanoislands were synthesized by high temperature annealing method with silver films under various processing conditions. Island parameters, such as size and separation, were analyzed using SEM images. Near-field distributions on nanoisland patterns were calculated by rigorous coupled-wave analysis and confirmed experimentally. The localization characteristics of SP on nanoisland patterns were analyzed in comparison with periodic nanostructures for SP based imaging. For live cell imaging, mouse macrophage-like (RAW) cell line, stained with Alexa Fluor 488, was prepared on the nanoisland patterns. Experimental results confirm the imaging resolution on the order of 100 nm. Lateral SP localization of nanoisland patterns also enhances image contrast. Various strategies to improve image sampling will also be presented. SP-enhanced microscopy based on a random distribution of nanoisland substrates is expected to provide a convenient way of imaging molecular interactions at subdiffraction-limited resolution for highly localized detection.

8954-14, Session 4

Nanoparticles as a theragnostic device for colorectal cancer *(Invited Paper)*

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No Abstract Available

8954-15, Session 4

High-sensitivity fluorescence imaging with micro/nanostructured terraces

Falco C. M. van Delft, Philips Research Nederland B.V. (Netherlands); Serban Dobroiu, Dan V. Nicolau, Univ. of Liverpool (United Kingdom)

No Abstract Available

8954-16, Session 4

Improving of enzyme immunoassay for detection and quantification of the target molecules using silver nanoparticles

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Modern routine enzyme immunoassays for detection and quantification of biomolecules have several disadvantages such as high cost, insufficient sensitivity, complexity and long-term execution. The surface plasmon resonance of silver nanoparticles gives reasons of creating new in the basis of simple, highly sensitive and low cost colorimetric assays that can be applied to the detection of small molecules, DNA, proteins and pollutants. The main goal of the study was the improving of enzyme immunoassay for detection and quantification of the target molecules using silver nanoparticles with hyaluronan. Principle of the new method lies in the ability of silver nanoparticles with hyaluronan to disintegrate into highly reactive nanoparticles in biological mediums, their re-bind with target molecules and formation of new detection complexes.

For this purpose we carried out size-controlled synthesis of silver nanoparticles with hyaluronan and studied their optical properties after the binding with target molecules. For the assessing of the new improving technique we used classic enzyme-linked immunosorbent assay test for determine of progesterone and estradiol concentration.

Our results showed that silver nanoparticles with hyaluronan were disintegrated with formation of new complexes in reactive medium. These bimolecular complexes had ability to light extinction and were detected by spectroscopy. The excess of free silver nanoparticles without target molecules aggregated into large clusters and lost their optical properties. Therefore, improved analytical techniques have great potential for detection of molecules and may be available tool for diagnosis of diseases.

8954-17, Session 4

Symmetries and biology: a new approach to biosensing

Mathieu L. Juan, Xavi Vidal, Gabriel Molina-Terriza, Macquarie Univ. (Australia)

We propose a novel approach to biosensing relying on symmetry considerations. In particular, we focus on the cylindrical symmetry: the proposed device can be rotated around a particular axis and still remain the same. The fundamental aspect of symmetry consideration gives rise to novel capabilities for biosensing: first the sensitivity is directly related to the impact the analyte has on the symmetry of the system and the reader capabilities, second no specific wavelength is imposed by such scheme.

For the system to fulfill cylindrical symmetry, we use a colloidal gold particle as sensor probed with an incident circularly polarized Gaussian beam. Owing to the symmetry of the system an important quantity is conserved: the total angular momentum. A deformation or the adding of a defect on the colloidal particle is detected by analyzing the field pattern after interaction. Physically, this modification of the pattern is related to the apparition of additional angular momentum components. By constantly analyzing the pattern of the scattered field, the bindings events are temporally resolved. In addition, as this method relies on geometrical considerations, the radial position of the adsorbed analyte can be deduced form the scattered field pattern.

As this approach relies on geometrical consideration, it provides important properties such as the possibility to multiplex spatially or in wavelength. In addition, it relaxes strongly the restriction on the sensor as no specific plasmon resonance is necessary. We believe this works opens promising alternatives for the development of biosensors.

8954-18, Session 5

Towards a versatile technique for tracking nanoparticle-mucus interaction: a step on the road *(Invited Paper)*

Noha Nafee, Philipps-Univ. Marburg (Germany) and Alexandria Univ. (Egypt); Marc Schneider, Philipps-Univ. Marburg (Germany)

Respiratory mucus is one of the main barriers for nanoparticle-based pulmonary delivery systems. This holds true especially for lung diseases like cystic fibrosis, where a very tenacious thick mucus layer hinders particle diffusion to the lung epithelium or the target area. Typically, mean square displacement of particles is used for mobility evaluation. In contrast, our objective is to develop a feasible technique to track particle penetration as a prerequisite for efficient pulmonary nanotherapy. Therefore, particle diffusion in artificial mucus was monitored based on confocal laser scanning microscopy (CLSM) and particle-mucus interaction was observed. As pharmaceutical relevant and benign materials, solid lipid nanoparticles (SLNs) were prepared by hot-melt emulsification using glyceryl behenate and different stabilizing agents such as poloxamer-407, polysorbate-80, and polyvinyl alcohol (PVA). The diffusion of labelled SLNs in labelled artificial sputum representing CF-patient sputum was verified by 3D time laps imaging. Thus, the effect of coating, particle size and mucus viscosity on nanoparticle diffusion was studied. Using image analysis software “Image J”, the total fluorescent signal after 30 min in case of poloxamer-coated SLNs was 5 and 100 folds higher than polysorbate- and PVA-coated SLNs, respectively. Nevertheless, increasing mucus viscosity reduced the diffusion of polysorbate-coated SLNs by 10 folds. Studying particle-mucus interaction by CLSM can be considered a promising and versatile technique, yet, various experimental parameters are to be optimized towards a more standardized procedure and more statistically-relevant data.

8954-19, Session 5

Efficient antibody-antigen sensing platform using plasmon field effect transistor

Hossein Shokri Kojori, Univ. of Miami (United States); Juhyung Yun, The State Univ. of New York at Buffalo (United States); Younghun Paik, Univ. of Miami (United States); Joondong Kim, Kunsan National Univ. (Korea, Republic of); Sung Jin Kim, Univ. of Miami (United States) and Biomedical Nanotechnology Institute (United States)

Localized surface Plasmon Resonance (LSPR) is a nanoscale phenomenon which presents strong resonance associated with noble metal nanostructures. Since LSPR is sensitive to the change of local refractive index, it has a great potential as a sensor in biomedical applications. In order to enable efficient sensing and an integrated device structure, we have developed a Plasmon field effect transistor. Electrically isolated gold nanoparticles in the transistor structure produces plasmon resonance induced hot electrons to the channel of field effect transistor and they contributes to increase the drain current. Plasmon FET is capable of multiplexing and integration in fluidic channels and it enables highly sensitive detection and a wide sensor dynamic range. We demonstrated an antibody based sensor using plasmon FET technology. Also, antigen antibody bonding based detection by using an array structure of Plasmon FETs will be presented. Functionalized gold nanoparticles with specific antibody is able to catch only one specific antigen or protein which causes refractive index change around gold nanoparticles and it can be easily detected by Plasmon FET. This novel antibody based sensor has several advantages such as extremely small size for integration and multiplexing, no need of complex optical geometry and robust operation.

8954-20, Session 5

Core-shell quantum dots for biosensing

Junjie Zhu, Nanjing Univ. (China)

The highly luminescent and low toxic glutathione-capped core-shell Quantum Dots CdSeTe@ZnS–SiO2 quantum dots were synthesized via a microwave strategy, and further we assembly a multifunctional Fe3O4/SiO2/CdSeTe@ZnS-SiO2/polydopamine nanoprobes. In graded-gap CdSeTe@ZnS-SiO2 quantum dots (QDs) bilayers, an electrochemiluminescence (ECL) enhancement effect was observed and used for ultrasensitive immunoassay. The CdSeTe@ZnS-SiO2 QDs with two different sizes were used as donor-acceptor pair because of their tunable energy and low biotoxicity. The graded-gap QDs bilayers were further used in the fabrication of ECL biosensor for the detection of carcinoembryonic antigen (CEA). We have also developed Fe3O4/SiO2/CdSeTe@ZnS-SiO2/polydopamine nanoprobe with strong fluorescence and fast magnetic response for specifically recognizing, fluorescently labeling, and magnetically sorting target tumor cells on a microfluidic chip. The outer polydopamine layer not only effectively alleviated the quenching effect to the interlayer quantum dots, but also provided a convenient and versatile functional interface to readily conjugate with the recognizing model molecules of aptamer KH1C12 with amine, thiol or carboxyl groups. Moreover, the polydopamine isolation and PEG decoration equipped the as-fabricated nanoprobes with little cytotoxicity and nonspecific affinity, leading to the effective and specific profiling of the protein epitopes expressed on the target tumor cells. The modified nanoprobe was utilized to label and isolate HL-60 cells from a homogenous cell mixture of HL-60 and K562 cells on a microfluidic chip. Combining with the high throughput of the microfluidic chip, 1.0 ? 104 HL-60 cells were readily separated from 2.0 ? 104 cells in only 10 min with 98% separation efficiency.

8954-21, Session 5

Advanced photonic crystal structures for enhanced sensitivity of biosensing devices

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In recent years, photonic crystal (PhC) structures showed a good potential for label-free biosensing [1]. These specially fabricated periodic nanostructures in which the dielectric constant periodically varies across the device can be specially designed to localize the light wave in the low refractive index detection region, which makes them highly sensitive to a small refractive index change produced by biological species.

Here we present a theoretical study of specially designed Si based 2D PhC waveguide structures with enhanced sensitivity to the refractive index change. Finite-Difference-Time-Domain (FDTD) simulations were employed to obtain transmission properties and a field distribution inside the waveguides. Studied structures were based on the W1-type PhC slab, where one row of holes was removed in the Gamma-K direction to create the waveguide. The lattice constant and the hole radius were chosen to obtain the upper edge (in terms of wavelength) of the guided band around 1700 nm. To increase the light confinement inside the detection area and so the band edge shift upon the refractive index change, additional row of holes and/or squares shifted to the crystal with the same periodicity were added into the waveguide. FDTD simulations were performed for various combinations of the hole radius, lattice constant and size of additional holes and squares. Theoretical results confirmed that the additional “sub-structures” in the PhC waveguide significantly improve its sensitivity to the refractive index change. This is very important form the application point of view.

8954-22, Session 5

Quantum dot microarrays for analyte sensing and cellular dynamics

Mihaela Delcea, Raghavendra Palankar, Nikolay Medvedev, ZIK HIKE, Univ. of Greifswald (Germany)

Quantum dot (QD) based micropatterned arrays are of broad interest in applications ranging from electronics, photonics, to sensor devices for biomedical purposes.1 Here, we report on a rapid, physico-chemically mild approach to generate high fidelity micropatterned arrays of pre-functionalized water soluble quantum dots using electron beam lithography (EBL). Such patterns retain their fluorescence and bio-affinity upon electron beam lithography and, based on the streptavidin-biotin interaction, allow for detection of proteins, colloidal gold nanoparticles and magnetic microparticles.2 QD-based microarray patterns differing in their shape (circles, squares, grid-like), size (from 1 to 10 ?m) and pitch distance are used to study the adhesion, spreading and migration of human blood derived neutrophils. We show using live cell confocal fluorescence microscopy that pattern geometry and pitch distance influence the adhesion, spreading and migratory behavior of neutrophils.2 In addition, we use QD nano-/microarrays to investigate the dynamics of blood platelets in heparin-induced thrombocytopenia (HIT). In HIT, antibodies are generated against multimolecular complexes formed by platelet factor 4 (PF4) proteins with heparin. The formed immune complexes activate platelets by cross-linking their Fc gamma receptor IIa (Fc?RIIa) leading to thrombocytopenia. To elucidate the mechanism involved therein at single cell regimes, we immobilize immune complexes and bimolecular components that induce HIT on hybrid microarrays of QDs fabricated using EBL.3 For the biophysical characterization of cellular responses, we employ live cell 5D quantitative imaging, total internal reflection fluorescence (TIRF), atomic force microscopy (AFM), quartz crystal microbalance (QCM), elipsometry and electron microscopy techniques. Our QD micropatterned arrays provide a tool for analyte sensing and cellular dynamics.

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8954-23, Session 6

Improved performance of highly multiplexed silicon-on-insulator microring sensor chips by surface structure implementation

Sam Werquin, Peter Bienstman, Ghent Univ. (Belgium); Diedrik Vermeulen, Acacia Communications (United States); Arne Goes, Agrosafve NV (Belgium); Anabelle Van Eeghem, Peter Dubruel, Ghent University (Belgium)

Silicon-on-insulator microring resonators have proven to be an excellent platform for label-free nanophotonic biosensors. The high index contrast of the silicon-an-insulator platform allows for fabrication of micrometer size sensors and a high degree of multiplexing. To enable robust, low-noise performance of a microring resonator sensor chip in a lab-on-a-chip setting, flood illuminating an array of vertical grating couplers is a promising approach to couple input light into the chip. This technique provides a very high alignment tolerance while at the same time exciting multiple sensors simultaneously for rapid parallel read-out. We demonstrate this technique to obtain a highly multiplexed chip output combined with real time sensor information. However, parasitic reflections on the chip surface can deteriorate the sensor signal and limit the performance. We investigate the use of surface structures to limit

these parasitic signals and show a significant improvement of the sensor operation.

8954-24, Session 6

Amplification and modulation of fluorescent signals by using hybridization chain reactions for multiplexed sensing of biomolecules in a one-pot

Takahiro Nishimura, Yusuke Ogura, Kenji Yamada, Yuko Ohno, Jun Tanida, Osaka Univ. (Japan)

Fluorescence readout of biomolecular information is a promising approach for biomolecular sensing. Control of fluorescence resonance energy transfer (FRET) by use of DNA nanostructures is useful to modulate fluorescent signals. In this study, we propose a biomolecular sensing method based on amplification and encoding of biomolecular signals by control of FRET with DNA reactions. Use of fluorescent signals represented by intensities for a set of wavelengths would offer a multiplexed biomolecular sensing that is carried out in a one-pot without micro-fabrication like DNA microarray. For the purpose, we designed a DNA reaction system based on hybridization chain reactions (HCR) that make long double-stranded DNA polymers. In the designed HCR, a target molecule triggers to start the HCR in which hairpin-structured DNAs labeled with both a fluorescent and quencher dye assemble the polymer structures. The fluorescent molecules in the absence of the targets are near the quenchers and the output fluorescence is suppressed by FRET. The polymerization process separates the fluorescent and quencher dye and the fluorescent signal increase. In experiments, a HCR system that detects a single-stranded DNA (24 nt) was prepared. The experimental results demonstrated that the fluorescent signal was amplified and the amplify ratio was modulated by composition ratio of the labeled hairpin and non-labeled one. We also confirmed that two different fluorescent molecules (FITC: green, TAMRA: red) were available for fluorescent readout by different color channels. This result leads to the multiplexed sensing in a one-pot by fluorescent amplification and multiple fluorescent color-coding.

8954-26, Session 6

Polymer slab waveguides for the optical detection of nanoparticles in evanescent field based biosensors

Nuria Teigell Beneitez, Univ. Gent (Belgium); Jeroen Missinne, Ghent Univ. (Belgium); Jean Schleipen, Joke Orsel, Menno Prins, Philips Research Nederland B.V. (Netherlands); Geert Van Steenberge, Ghent Univ. (Belgium)

We present a polymer optical waveguide integration technology for the detection of nanoparticles in an evanescent field based biosensor.

In the proposed biosensor concept, superparamagnetic nanoparticles are used as optical contrast labels. The nanoparticles capture target molecules from a sample fluid and bind to the sensor surface with biological specificity. The surface-bound nanoparticles are detected using frustration of an evanescent field.

In the current paper we describe polymer waveguides which are used to generate a well-defined optical field for nanoparticle detection. The planar waveguide approach offers integration advantages and possibilities for multiplexing; in addition, polymers are suited for cost-effective fabrication.

The generation of the surface localized evanescent optical field is achieved by employing polymer slab waveguides. Several waveguide configurations are explored using Finite Difference Time Domain (FDTD) simulations and then experimentally verified. The main design parameters are signal intensity and penetration depth of the optical field at the waveguide top surface. The waveguides are characterized in terms of fabrication quality (dimensions, surface roughness) and also optically by determining the waveguide loss and by visualizing the mode profiles at the waveguide end-face using an optical near-field profiler. Finally, the waveguide substrates are combined with microfluidic channels and we demonstrate the detection of surface-bound nanoparticles at the sensor surface.

8954-27, Session 7

Surface-enhanced Raman scattering (SERS) for detection of phenylketonuria for newborn screening

Mehdi Javanmard, Ronald Davis, Stanford Univ. (United States)

Diagnosis of Phenylketonuria (PKU) in newborns is important because it can potentially help prevent mental retardation since it is treatable by dietary means. PKU results from a deficiency of phenylalanine hydroxylase, an enzyme that catalyzes hydroxylation of phenylalanine to tyrosine, thus resulting in phenylketonurics having phenylalanine levels as high as 2 mM whereas the normal upper limit in healthy newborns is 120 uM. To this end, we have developed a microfluidic platform integrated with a SERS substrate for detection of high levels of phenylalanine.

As a proof of concept, we have successfully demonstrated SERS detection of phenylalanine using various SERS substrates. We have explored the use of both gold nanorod and also nanosphere lithograph, both of which exhibit high levels of field enhancement. Using this technique we have demonstrated phenylalanine detection at clinically relevant levels. In this presentation, we will discuss both the fabrication and characterization of the SERS substrate, and we will also discuss the microfluidic platform used for sample preparation.

8954-28, Session 7

Role of nonspecific binding: a comparison among flow through and flow over assays in nanoporous material

Paolo Bettotti, Neeraj Kumar, Univ. degli Studi di Trento (Italy); Romain Guider, University of Trento (Italy); Elena Froner, Marina Scarpa, Univ. degli Studi di Trento (Italy)

Nanoporous materials are ideal host to develop high sensitivity devices because of their large specific area and the possibility to tune the matrix-analyte interaction by modifying their structure and surface functionalization. A major problem of these material is the signal generated during the assay because of non specific binding. This is an important and general effect that is usually overlooked in nanopores based biosensing literature.

Here we compare flow over (FO) against flow through (FT) assay geometries and we demonstrate that porous biosensors fabricated in nanopores with closed ended pores sistematically overestimate the sensitivity of the device because of a poor removal of non bound analytes. We investigate different sets of samples and the reliability of our approach is tested against different types of assays (FO, simple diffusion and FT forced using a pump). Our experiments demonstrate that the mass transport through the nanopores is essential to provide a proper removal of not bounded analytes that otherwise contribute as non specific signal.

To our best knowledge this is the first time that such effect is quantified in nanoporous material and our work indicates a clear limit to the sensitivity of porous hosts used in FO geometry.

8954-29, Session 7

Three-photon fluorescence nano-thermometers based on intensity and spectral shift using Yb/Tm co-doped NaNbO3 nanocrystals

Kagola Upendra Kumar, Wagner Ferreira da Silva, Weslley Queiroz Santos, Carlos Jacinto da silva, Univ. Federal de Alagoas (Brazil)

Thermal sensing at the micro- and nanoscales is required for high spatial resolution of temperature gradients and is an indispensible tool for dynamical studies of diverse small systems including electrical, photonic, and biological ones. In the present work, we present a three photon fluorescence nanothermometer based on intensity and spectral shift dependences on the temperature using Yb/Tm co-doped sodium niobate nanocrystals, prepared by a sol-gel method. The emission spectra of 1Tm3+/5 Yb3+ codoped NaNbO3 exhibit three emission bands centered at 480, 650 and 800 nm upon 976 nm CW laser excitation at room temperature.

The upconversion emission at 480 and 800 nm are ascribed to the 1G4 ? 3H6 and 3H4 ? 3H6 transitions, respectively. The emission at 650 nm is ascribed to the 2F2,3 ? 3H6 and/or 1G4 ? 3F4 transitions, owing to stepwise absorptions of two or three excitation photons by the Yb3+, followed by energy transfer to the Tm3+.

A very interesting spectral shift was obtained with the temperature variation from 20 -80 oC. The shift show linear behaviour against temperaure with slope of (1.3 ± 0.1) cm-1/oC. If the emission wavelength is fixed, from normalized spectra we can note also the intensity temperature dependence. The origin of the spectral shift is being investigated. The explanation was given considering both the static contribution due to lattice thermal expansion and vibrational contribution due to electro-phonon interaction, but a deeper analysis is necessary. Anyway, the results are very interesting and deserve attention for possible applications mainly in biomedicine.

Conference 8955: Colloidal Nanoparticles for Biomedical Applications IX

Saturday - Tuesday 1 –4 February 2014

Part of Proceedings of SPIE Vol. 8955 Colloidal Nanoparticles for Biomedical Applications IX

8955-1, Session 1

Lipid-modified PAMAM dendrimers as a tool for the design of nanoparticle-based multimodal MRI contrast agents

Adriano Boni, Mauro Gemmi, Istituto Italiano di Tecnologia (Italy); Claudia Innocenti, Univ. degli Studi di Firenze (Italy); Giuseppe Bardi, Alice Bertero, Giovanni Signore, Angelo Bifone, Istituto Italiano di Tecnologia (Italy)

Magnetic nanoparticles are often synthesized by decomposition of organometallic precursors 1-3 into hot surfactant solutions, a technique that results in highly crystalline iron oxide cores with a narrow size distribution. In order to use these nanoparticles for biomedical applications, replacement of the hydrophobic coating with a hydrophilic one is necessary to obtain stable and injectable aqueous solutions. Among the available hydrophilic coatings, dendrimers, highly monodisperse hyperbranched polymers with a repetitive and perfectly defined structure, have attracted considerable interest.4-7

Recently, we presented a novel and facile method to attach commercially available lipid-modified PAMAM dendrimers to the surface of iron oxide nanoparticles in a single step in a water/pentane mixture at room temperature.8 Here we report the latest developments of this work.

By means of this platform, we have been able to tune the relaxometric properties of iron oxide nanoparticles, such as coating thickness and core size, to obtain the best relaxometric efficiency with a high degree of control. Also, we have performed in vitro biocompatibility tests and half-life in vivo measurements to demonstrate the possibility to use this materials as MRI negative contrast agents.

Finally, we have been able to modify the surface of the nanoparticles with gadolinium complexes in order to obtain a multimodal contrast agents which can be used to obtain either T1 and T2 weighted MRI images. The structural and relaxometric properties of this material will be discussed.

8955-2, Session 1

Encoded nanospheres as biomarkers for the ratiometric detection of cystic fibrosis *(Invited Paper)*

Iván Castelló-Serrano, Georgiana Stoica, Emilio J. Palomares, ICIQ - Institut Català d'Investigació Química (Spain)

Current detection technologies for cystic fibrosis diagnosis involves quantifying trypsinogen in the blood of the babies (immunoreactive trypsinogen test, or IRT), which typically makes use of fluorescent-based immunoassays. However, these tests are slow, costly, and still have the drawbacks with respect to specificity, time-effectiveness and user-friendliness. This procedure is long and sometimes considered invasive by the parents. We present herein two colour encoded silica nanospheres (2nanoSi) for the fluorescence quantitative ratiometric determination of trypsin in humans. The biomarker used in this work consist in a well-designed and cost effective nanosystem based on FRET with an internal reference that unequivocally permits the ratiometric determination of trypsin in the range of enzyme concentration relevant for clinical determination of cystic fibrosis in minutes, with high sensitivity. A short peptide substrate previously marked with TAMRA, a Rhodamine-derived dye, is anchored to the surface of a silica nanosphere containing two types of quantum dot nanocrystals with different fluorescence emission wavelengths. The 2nanoSi system proved to be a fast (minutes), non-invasive, a single-step and with two times higher sensitivity than the state-of-the-art biomarkers based sensors for cystic fibrosis, allowing the quantification of trypsin concentrations in a wide range (25-350 ug/L). Moreover, our approach can be used from the 4th day of life when the trypsin concentration is already the same as in adults.8 Furthermore, as trypsin is directly related to the development of cystic fibrosis (CF), different human phenotypes, i.e. normal (160-340 ug/L), CF homozygotic (0-90 ug/L), and CF heterozygotic (91-349 ug/L), respectively, were determined using our 2nanoSi nanospheres in real samples. We anticipate the 2nanoSi system to be a starting point for non-invasive, easy-to-use and cost effective ratiometric fluorescence biomarker for recessive genetic diseases alike human cystic fibrosis.

8955-3, Session 1

Toward efficient modification of large gold nanoparticles with DNA *(Invited Paper)*

Ron Gill, Kristian Göeken, Univ. Twente (Netherlands); Vinod Subramaniam, FOM Institute AMOLF (Netherlands)

DNA-coated gold nanoparticles, are one of the most highly researched nano-bio hybrids for biomedical applications. In addition to drug delivery and biosensing, they can also be used for other optical technologies such as the building of photonic crystals. A prerequisite for all these applications is the stable modification of DNA-coated nanoparticles. The classical modification methods first proposed by the group of Prof. Mirkin from Northwestern involves long incubation steps, with slow addition of salt in small steps, and for large particles also involves a large excess of the DNA compared to what can bind to the surface. Several groups have in recent years shows that using surfactants to stabilize the particles, either faster salting steps, or even putting high salt concentration from the start. Still using these methods with large (40nm+ diameter) gold nanoparticles have been difficult because of the lower stability of large particles. Here we present our result on a different approach toward fast modification of the nanoparticles – instead of stabilizing the nanoparticles against salt-induced aggregation, we reduce the negative charge of the DNA to increase the binding kinetics at lower salt concentrations. We will show results both with the acidic buffer modification methods proposed last year by the Liu group from the U. of Waterloo, and also recent results we got with DNA modified with a polycationic tail.

8955-4, Session 1

Conjugated polymer nanoparticles as biological imaging agents *(Invited Paper)*

Mark A. Green, King's College London (United Kingdom)

Fluorescent nanoparticles have captured the interest of scientist in many aspects, they have found applications in photo-electronics such as LEDs, and in the biological and medical fields such as bio-imaging and bio-tracking. Many efforts were reported in the synthesis of highly-fluorescent inorganic nanoparticles in the form of quantum dots (QDs). However, QDs have many health issues related to their composition, and even when capped, their use in-vivo will always be a concern. Therefore, other alternatives were brought out where benign, organic materials, such as conjugated polymers, were used to form fluorescent nanoparticles with competing properties. Bifunctional flourescent nanoparticles have also been introduced as potential MRI contrast agents with the incorporation of iron oxide nanoparticles in their cores or with the association of gadolinium atoms to their surfaces. The Gd-bifunctional SPNs were found to have smaller diameters, with diameters comparable to similar surface coated QDs as suggested by both TEM and dynamic

light scattering (DLS). After an excessive purification process, the concentration of gadolinium in the SPNs system was determined by mass spectroscopy, and the MRI longitudinal (T1) relaxation times for a series of dilutions were measured resulting in a relaxivity of 10.9 mM-1.s-1.

8955-5, Session 2

Characterization of single gold nanoclusters

Dorota Buczynska, Lukasz Bujak, Nicolaus Copernicus Univ. (Poland); Fadi H. Aldeek, Hedi Mattoussi, Florida State Univ. (United States); Sebastian Mackowski, Nicolaus Copernicus Univ. (Poland)

Gold nanoclusteres (AuNC) are small, ~1.2 nm in size, metal core capped with a ligand. In our case the ligand was lipoic acid appended with polyethylene glycol. In this work we describe the fluorescence properties of these nanostructures with particular emphasis on their unique emitting properties. In order to assess the fluorescence characteristics of the AuNCs we employ single molecule spectroscopy and microscopy approaches.

The NCs were dispersed in either water or toluene and further diluted to achieve appropiate, ultra-low concentrations for observation of single nanoclusters. Acquired intensity and size distributions of single fluorescent spots on fluorescence images indicate that we achieve single cluster emission in our samples. The samples were prepared by spin-coating prepared solutions on glass coverslips. Fluorescence properties of the clusters were studied using wide-field fluorescence microscopy and confocal microscopy.

While classical emitters, such as organic dyes or semiconductor quantum dots feature intensity jumps and step-wise photobleaching, the emission of single AuNCs exhibit continuous decrease of intensity. Our observations indicate that the presence of the ligand and interactions associated with it strongly modify the emission properties of the AuNCs. The fluorescence properties of the AuNCs depend on the polymer matrix and excitation. In particular AuNCs embedded in PVA are less sensitive to photobleaching. On the other hand, photobleaching is more efficient for the excitation at 405 nm as compared to AuNCs excited with 485 nm. We find that fluorescence intensity was lower for AuNCs embedded in PVA as compared to those in PMMA matrix.

8955-6, Session 2

Impact of solvent mixture solvents on iron nanoparticles generated by laser ablation

Mbarek Chakif, Andreas Ostendorf, Evgeny Gurevich, Ruhr-Univ. Bochum (Germany)

Laser ablation in liquid (LAL) is a promising method to generate nanoparticles of different materials because it allows to use a broad range of target materials and thus, can address different application area. Moreover, it is quite simple to control the nanoparticles properties by changing the laser parameters. Particularly, iron nanoparticles with specific magnetic properties and good biocompatibility are of interest in medical applications. A method will be described for the generation and investigation of phase-controlled iron oxide magnetic (FexOy) nanoparticles from iron-bulk target using femtosecond laser pulses with different ratio of water and ethanol as the solvent. X-ray diffraction (XRD) measurements revealed the nanoparticles constitution with either Fe or Fe2O3 composition. In Vibrating Sample Magnetometer (VSM), iron nanoparticles show different magnetic properties. The samples exhibit different coercivity and remanence with, respect to the experimental parameters. Furthermore, transmission electron microscope (TEM) images show homogeneously core-shell structure with a crystalline Fe core. In addition, spherical shape can be observed. In all samples a polydisperse particle size distribution (diameter 5-160 nm) and different mean diameters could be observed. Moreover, laser ablation in water and ethanol yields agglomerates, whereas in a mixture of both solvents a chain structures were formed.

8955-7, Session 2

Stabilization and size control of ligand-free gold and alloy nanoparticles in the presence of highly-diluted electrolytes *(Invited Paper)*

Christoph Rehbock, Vivian Merk, Lisa Gamrad, Jurij Jakobi, Univ. Duisburg-Essen (Germany); Daniela Tiedemann, Ulrike Taylor, Wilfried Kues, Detlef Rath, Friedrich-Loeffler-Institut (Germany); Stephan Barcikowski, Univ. Duisburg-Essen (Germany)

In order to correctly assess toxicological effects of nanoparticles released from coated implants, testing systems with high purity are required. Unfortunately, nanoparticles obtained from chemical synthesis are contaminated with artificial ligands, which may interfere with toxicity assays [1]. In order to overcome these limitations, ligand-free colloidal nanoparticles may be obtained by pulsed laser ablation in liquid [2]. Main drawback of this method, however, is control of the particle size. To this end the influence of inorganic salts is highly interesting, as they are ubiquitous in all biological systems.

In this work the impact of electrolytes on ligand-free gold and gold-silver alloy nanoparticles, is studied in the Hückel regime at extremely low ionic strengths. Interestingly, an anion specific stabilization of the colloids was found and could be correlated to the polarizability of the corresponding ion following a Hofmeister series. Additionally, a size quenching of the fabricated nanoparticles dependent on the ionic strength present during synthesis was found and verified by analytical disk centrifugation (ADC) and electron microscopy. This allowed size control of the nanoparticles in a size regime from 6-20 nm and a reduction of the particle size distribution [3]. The thus optimized NPs were applied in toxicity assays with gametes, where their impact on sperm motility, fertilization and embryo development was examined.

[1] Uboldi et al., Particle and Fibre Toxicology 6:18 (2009)

[2] Petersen, Barcikowski., J. Phys.Chem.C 113, 19830-19834 (2009)

[3] C. Rehbock, V. Merck, L. Gamrad, R. Streubel, S. Barcikowski, Phys. Chem. Chem. Phys 2013, 15, 3057 -3067

8955-8, Session 2

Controlled synthesis of gold nanoaggregates

Desiree F. Van Haute, Jacob M. Berlin, City of Hope Beckman Research Institute (United States)

Gold nanoaggregates assembled from individual gold nanoparticles have been studied in ex vivo materials applications for their plasmonic properties, including surface enhanced Raman scattering, and potential in chemical sensing. These properties of nanoaggregates could be useful in vivo for diagnostic, therapeutic or imaging modalities, but current synthetic methods generally lead to ill-defined nanoaggregates or required sophisticated polymer additives. Additionally current methods rely heavily on organic solvents to produce aggregates, rendering them poorly biocompatible, and the aggregates are difficult to handle due to reactive surfaces. We have developed a straightforward and modular synthetic method to create and cap gold nanoaggregates to enable their use in aqueous, organic and biological environments. Individual nanoparticles 5-20 nm in size can be controllably assembled to produce aggregates ranging in size from 15 – 100 nm. The properties of the nanoparticles aggregates are controlled by selection of capping agent. Capping with a phenyl or PEG layer renders the resulting stable aggregates organically soluble or biocompatible, respectively. Fluorescent labels were also readily introduced using this strategy and efficient and non-toxic uptake into cells was observed. These aggregates hold great promise for in vivo imaging and potentially drug delivery.

8955-9, Session 2

Design of bivalent gold nanoparticle-oligonucleotide-peptide conjugates for duplex and triplex hybridization

Lisa Gamrad, Anna Ziefuß, Christoph Rehbock, Stephan Barcikowski, Univ. Duisburg-Essen (Germany); Ulrike Taylor, Roberto Mancini, Detlef Rath, Friedrich-Loeffler-Institut (Germany)

Bioconjugated gold nanoparticles (AuNPs) are frequently used in medical diagnostics and therapy, e.g. in bioimaging [1]. For cell selective imaging applications, nanoparticles have to be translocated across the plasma membrane and need to bind selectively inside the cell. To this end, bivalent conjugates containing cell penetrating peptides (CPPs) to transport nanoparticles into cells and specific binding molecules like oligonucleotides are required. These biomolecules can be bound to AuNPs via a thiol group. In this context, high ligand loads and a controlled surface coverage are very important for an efficient nano-bio-interaction. Therefore, ligand-free AuNPs obtained by pulsed laser ablation in liquid were used. They are known to have a five times higher surface coverage during bioconjugation compared to chemically synthesized particles functionalized by ligand exchange [2].

This work focuses on the design of bivalent bioconjugates with oligonucleotides and CPPs and elucidates the influence of a second ligand. Therefore, laser-generated AuNPs with a defined size (5nm), reached by size quenching effects due to inorganic salts [3], were sequentially ex situ conjugated. Conjugates were assessed according to their surface coverage, aggregation tendencies, size and charge after every conjugation step. In order to test specific binding capacities of surface-bound oligonucleotides, they were subjected to triplex hybridization experiments with corresponding DNA-sequences. In this context, the efficiency of this process in the presence of AuNPs and CPPs was thoroughly examined.

[1] Qian et al., Nat. Biotech., 26,1 (2008)

[2] Petersen et al., Phys. Chem. C., 113, (2009)

[3] Rehbock et al., Phys. Chem. Chem. Phys., (2012)

8955-10, Session 3

Multidentate oligomeric ligands to enhance the biocompatibility of iron oxide and semiconductor nanoparticles

Wentao Wang, Goutam Palui, Xin Ji, Fadi H. Aldeek, Hedi Mattoussi, Florida State Univ. (United States)

We have developed a new versatile surface-functionalization strategy based on ligand exchange that is applicable to metal oxide nanoparticles (e.g., Fe3O4 NPs). This strategy involves the design of new set of multidentate dopamine oligomers prepared using one-step reaction of a polymer backbone with lateral poly(ethylene glycol) short chains (or other hydrophilic moieties), and dopamine anchors. Here the catechol groups allow strong affinity to the iron oxide NPs, while dispersion in buffer media and biocompatibility is promoted by the PEG moieties. Ligand exchange of Fe3O4 NPs with these oligomers is remarkably efficient compared to other mono-coordinating capping molecules. The resulting NPs preserve their homogenous size and distribution and exhibit high colloidal stability over a broad range of conditions. Furthermore, our strategy allows the easy insertion of a controllable number of functional groups (e.g., amine or azide) on the NPs, making them compatible with commonly used bio-orthogonal chemistries. We also found that by substituting dopamine with other polar groups, such as lipoic acid, provides polymer ligands that are very effective for functionalizing QDs and Au Nanoparticles.

8955-11, Session 3

Gold nanorods as multifunctional agents in DNA systems

Joanna Olesiak-Banska, Marta Gordel, Katarzyna Matczyszyn, Wroclaw Univ. of Technology (Poland); Joseph Zyss, Ecole Normale Supérieure de Cachan (France); Marek J. Samoc, Wroclaw Univ. of Technology (Poland)

Gold nanorods (NRs) present unique optical properties due to their tunable surface plasmon resonance in the NIR region as well as their photothermal properties [1]. Moreover, the nonlinear optical phenomena in NRs can be observed in the wavelength range where strong one-photon absorption and absorption saturation is present, in addition to resonant two-photon excitation [2]. In this work, we combine the luminescent and thermal properties of gold NRs to image and manipulate self-assembled DNA structures on the nanometer scale. We prepared DNA lyotropic liquid crystal phases (LLC), which can be considered as a model of DNA organization in vivo. DNA strands organization in the LLC phases can be visualized with several microscopy techniques, but we proved that polarization-sensitive two-photon fluorescence microscopy (ps-2PFM) has multiple advantages and provides full information about DNA order in three dimensions [3,4]. Here, we show that gold NRs can serve as luminescent probes for ps-2PFM and present the polarization analysis of 2PL of a single gold NR in the DNA matrix. Illumination of a single NR with a laser beam results in local heating. As the DNA LC phases depend strongly on temperature, we also characterize the photothermal effects in the DNA-NRs system and discuss the application of the NRs for the control of local organization of DNA.

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[2] J. Olesiak-Banska et al., J. Phys. Chem. C, 116 (2012), 13731-13737

[3] H. Mojzisova et al., Biophys. J. 97 (2009), 2348-2357

[4] J. Olesiak-Banska et al., Biopolymers, 95 (2011), 365-375

8955-13, Session 3

Multidentate polymeric ligands for long-term bioimaging using highly stable and functionalized quantum dots

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Colloidal fluorescent semiconductor nanocrystals, named “quantum dots”, possess unique features, such as a tunable peak wavelength (according to their composition and their size) or a large absorption cross-section, that make them very attractive for biomedical imaging. Nevertheless, typical syntheses provide nanoparticles capped with hydrophobic ligands. To be used in long-term bioexperiments, they have thus to be modified to exhibit essentially a high colloidal stability in aqueous conditions, but also a low non-specific adsorption, a small size and functionalization moities. As all of these properties are controlled by the layer of coating ligands, we designed a bidentate monozwitterionic ligand, to first address the need of small-sized and antibiofouling hydrophilic probes. But the corresponding quantum dots revealed to be unstable in highly diluted conditions and difficult to functionalize. To further increase the affinity between the nanoparticles and their surrounding ligands, we synthesized a multidentate polyzwitterionic ligand, issued from the copolymerization of a bidentate monomer and a monozwitterionic one. The nanocrystals passivated by this polymeric

ligand showed an exceptional colloidal stability, regardless of the medium conditions (pH, salinity, dilution, and biological environment), and we demonstrated the affinity of the polymer exceeded by three orders of magnitude that of the bidentate ligand. The synthesis of the multidentate polyzwitterionic ligand proved also to be easily tunable and allowed the facile introduction of reacting moieties. Further functionalization of the corresponding quantum dots with biomolecules led to successful specific targeting, which could be confirmed, as an example, through FRET experiments.

8955-14, Session 4

Peptide-modified gold nanoparticles for improved cancer therapeutics *(Invited Paper)*

Celina Yang, Mehrnoosh Neshatian, Devika B. Chithrani, Ryerson Univ. (Canada)

The application of nanoparticles (NPs) for improved therapeutics is at the forefront of cancer nanotechnology. Among other NP systems, gold nanoparticles (GNPs) are extensively used due to its impressive ability to act as both an anticancer drug carrier in chemotherapy and as a dose enhancer in radiotherapy. GNPs used in those studies were predominantly localized in the cell cytoplasm. However, the therapeutic response can be further enhanced if NPs can be effectively targeted into the nucleus. Here, we present an effective strategy for designing a GNP-peptide complex for nuclear targeting. Two peptides were conjugated onto a GNP: One peptide enhanced the uptake while the other peptide enhanced the nuclear delivery. The nuclear targeted cells displayed a four–fold increase in the therapeutic response when treated with radiation as compared to untargeted ones. There was a modest increase in the DNA damage for radiated cells with nuclear targeted GNPs. This research will establish a more successful NP-based platform for combining more than one treatment modality, such as chemotherapy and radiotherapy, and creates a more aggressive approach in eradicating cancer.

8955-15, Session 4

Controlling the orientation of DNA-assembled gold nanorods

Jessica M. Smith, Leslie Hamachi, Vivian E. Ferry, Univ. of California, Berkeley (United States); Young-Wook Kim, Univ. of California, San Francisco (United States); Somin E. Lee, Lawrence Berkeley National Lab. (United States); Paul Alivisatos, Univ. of California, Berkeley (United States)

Gold nanorods are widely used for imaging applications because of their strong which is tunable into the infrared. The scattering frequency of a gold nanorod is commonly tuned by changing the size or aspect ratio of the nanorod. The optical properties of nanorods can also be manipulated by bringing the rods in proximity in a controlled way. Toward this goal, our lab has developed a process by which short strands of DNA can be used to precisely spatially order gold nanoparticles. Recent research has focused on the incorporation of anisotropic (non-spherical) nanoparticles to tailor more complex resonances in these DNA-assembled structures. We recently demonstrated for the first time that gold nanorods can be assembled by this method.

We have control over the relative orientation of the nanorod pairs, creating a plasmon ruler in the IR for biosensing applications. To create orientation controlled DNA-assembled nanorods, oligonucleotides are conjugated to the gold surface of a nanorod via a 5' branched trithiol moiety. Singly conjugated nanorods are separated from other products using anion exchange HPLC. Complementary conjugates are reacted; dimers are separated using agarose gel electrophoresis. By carefully tuning the conditions of separation, a sample of gold nanorod dimers that are attached side-by-side can be isolated from those attached end-to-end. These oriented pairs can be cut using a DNA restriction enzyme, EcoRV, demonstrating their utility as an ensemble biosensor.

8955-16, Session 4

Synthesis of metallic magnetic nanorods protected by noble metal shell *(Invited Paper)*

Katerina Soulantica, Sergio Lentijo-Mozo, Teresa Hungria-Hernandez, Benoit Cormary, Reasmey P. Tan, Pier-Francesco Fazzini, Marc Reaspaud, Univ. de Toulouse (France); Christophe Gatel, Ctr. d'Elaboration de Matériaux et d'Etudes Structurales, CNRS (France)

Cobalt nanorods are ferromagnetic at room temperature due to their high magnetocrystalline and shape anisotropy. They are good candidates for applications requiring "hard" magnetic nanoparticles (information storage, permanent magnets), however their use as in vitro biodiagnostic probes is compromised by their air sensitivity which transforms them to cobalt oxide with a concomitant deterioration of their advantageous magnetic properties. The development of a continuous noble metal shell around the cobalt cores would be an ideal solution for rendering the nanorods appropriate for use in an aqueous environment. The growth of a full shell is not straightforward, since segregation of the noble metal on the surface of cobalt results to noble metal decorated nanorods and not to a continuous full shell. However, even small discontinuities are enough for cobalt oxidation to take place in an aqueous solution. The introduction of an intermediate buffer layer between the magnetic core and the noble metal is a key step for the development of a full noble metal shell. Indeed the compatibility of the intermediate layer with both core and shell materials allows the formation of energetically favorable interfaces that are not disrupted. After noble metal coating, the nanorods are air and water resistant and can be transferred to an aqueous solution and be functionalized by standard antibody immobilization protocols without losing their magnetic properties.

8955-17, Session 4

Temperature: the "ignored" factor at the nano-bio interface

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Upon incorporation of nanoparticles (NPs) into the body, they are exposed to biological fluids, and their interaction with the dissolved biomolecules leads to the formation of the so-called protein corona on the surface of the NPs. The composition of the corona plays a crucial role in the biological fate of the NPs. While the effects of various physico-chemical parameters on the composition of the corona have been explored in depth, the role of temperature upon its formation has received much less attention. In this work, we have probed the effect of temperature on the protein composition on the surface of a set of NPs with various surface chemistries and electric charges. Our results indicate that the degree of protein coverage and the composition of the adsorbed proteins on the NPs surface depend on the temperature at which the protein corona is formed. Also, the uptake of NPs is affected by the temperature. Temperature is, thus, an important parameter that needs to be carefully controlled in quantitative studies of bio-nano interactions.

8955-18, Session 4

Delivery of tobramycin coupled to iron oxide nanoparticles across the biofilm of mucoidal Pseudonomas aeruginosa and investigation of its efficacy

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One mechanism of antibiotic drug resistance is the production of extracellular protective polymer matrices (biofilms) produced by pathogenic bacterial colonies in response to anoxia, low nutrient conditions, or in the presence of antibiotic drugs or antibodies. These polymers mechanically block antibiotic drugs from interacting with the bacterial colonies. We have investigated the effectiveness of magnetic hyperthermia and magnetic gradient-guided drug delivery for the treatment of pathogenic antibiotic-resistant Pseudomonas aeruginosa biofilm communities using biofunctionalized aqueous ferrofluids, consisting of superparamagnetic iron oxide nanoparticles (SPIONs) conjugated to tobramycin. Tobramycin is the antibiotic of choice in treating cystic fibrosis patients infected with P. aeruginosa. However, the effectiveness of aerosol treatment is limited by the biofilm barrier. Our previous experiments were conducted with planktonic P. aeruginosa. Here, we show that coupling tobramycin to SPIONs is a viable option for the treatment of P. aeruginosa biofilm colonies; surpassing antibiotic treatment alone.

8955-19, Session 4

Magnetically triggered active hybrid-nanocomposites

Marc Behl, Muhammad Y. Razzaq, Karl Kratz, Andreas Lendlein, Helmholtz-Zentrum Geesthacht (Germany)

Magnetically active materials have a wide application potential, e.g. in medical technology, where remotely controlled catheters or drug delivery systems could be thought of. A non-contact triggering of shape changes was realized by incorporating magnetic nanoparticles into shape-memory polymers.[1,2] These nanocomposites are heated inductively when they are exposed to alternating magnetic fields. Once a switching temperature is exceeded the recovery of a permanent shape is induced. Such nanocomposites can be designed multifunctional by a combination of different heating sources, which allows to adjust the apparent switching temperature (Tsw,app) of these polymers. Tsw,app is determined as the environmental temperature at which the shape-memory effect occurs. Weak alternating fields as an additional heating source reduce Tsw,app. [4] Beyond that, the capability of magnetic nanocomposites to remember the magnetic field strength (H) at which they were deformed before has been described as magnetic-memory effect. Here a temperature memory polymer is used as stimuli-sensitive matrix.[5] However, homogenous distribution of magnetite nanoparticles in crystallizable matrices is a challenge. A more homogenous distribution of the magnetite nanoparticles can be achieved by a decoration of the particles with a polymer shell.[6] Magnetic particles decorated with a crystallizable matrix can be obtained by the ring-opening polymerization of cyclic lactones onto the particle surface.[5] Furthermore, these decorated nanoparticles can be used as covalently integrated netpoints of hybrid nanocomposite networks. Upon application of an alternating magnetic field these hybrid nanocomposites were capable of a reversible movement as the covalent integration helped to reach a bulk temperature (Tmax) higher than the melting temperature (Tm) at a lower particle content compared to composites with non-covalently integrated nanoparticles. [7] In this way these hybrid nanocomposites might pave the way novel magnetic actuators.

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8955-20, Session 5

Optical properties and surface chemistry of quantum dots, polymeric and upconversion nanoparticles *(Invited Paper)*

Soheil Hatami, Martin Kaiser, Christian Würth, BAM Federal Institute for Materials Research and Testing (Germany); Susanne Leubner, Technische Univ. Dresden (Germany); Thomas Behnke, Marko Moser, Ralf Schneider, BAM Federal Institute for Materials Research and Testing (Germany); Nikolai Gaponik, Alexander Eychmüller, Technische Univ. Dresden (Germany); Ute Resch-Genger, Bundesanstalt für Materialforschung und -prüfung (Germany)

Fluorescent particles like nm- and ?m-sized polymeric beads doped or labeled with different types of fluorophores and nanocrystalline systems like quantum dots and upconversion phosphors emitting in the visible (vis), near-infrared (NIR), and IR (infrared) region are of increasing importance as fluorescent reporters for bioanalysis and medical diagnostics [1,2]. The assessment and comparison of material performance as well as the development of rational design strategies for improved systems require spectroscopic tools for the determination of their signal-relevant optical properties like their fluorescence quantum yields and brightness values [3-7] and analytical tools, which enable the determination of the number of surface groups, ligands, and /or fluorophores per bead [8-10].

This encouraged us to built up two integrating sphere setups for absolute measurements of fluorescence quantum yields of liquid and solid, transparent and scattering materials in the wavelength region from 350 nm to 1600 nm. In addition, a setup was developed for excitation power density dependent measurements of upconversion fluorescence quantum yields. In parallel, simple strategies for the characterization of the surface chemistry of different types of nanomaterials like polymeric beads and quantum dots were investigated and validated [9,10]. Here, we present different examples for the optical and analytical characterization of nanoparticles employed as fluorescent reporters and nanosensors and for ?m-sized beads used as platform for fluorescence assays.

8955-21, Session 5

Nonthermal damage caused by shining light on intracellular plasmonic nanoparticles *(Invited Paper)*

Samantha Chadwick, Martin Volk, Univ. of Liverpool (United Kingdom); Ian A. Prior, Univ of Liverpool (United Kingdom); Mathias Brust, Univ. of Liverpool (United Kingdom)

Hyperthermia caused by plasmonic heating is a promising approach to the future therapy of some cancers. There is now increasing evidence, that besides heating, also non-thermal, photochemical processes are involved that may cause significant damage and cell death, This presentation focusses on these effects and seeks to assemble a more complete mechanistic picture on the various factors that cause damage to sub-cellular structures when cells are illuminated by laser light in the presence of intracellular plasmonic nanoparticles. This presentation will summarize our own work over the past four years on non-thermal

cell death under laser illumination and it will also draw on a host of resent publications by others on photochemistry coupled to plasmonic nanoparticles. Finally, photochemical pathways to free radicals are suggested as an explanation for non-thermal cell damage and other photochemical chemical effects observed. The role of non-thermal effects will be discussed both in the absence and in the presence of significant plasmonic heating.

8955-22, Session 5

Colloidal hybrid metal oxide nanoparticles: from in silico to in vivo: cellular imaging quantification and biomedical applications *(Invited Paper)*

Marie Helene Delville, Institut de Chimie de la Matière Condensée de Bordeaux-CNRS (France) and CENBG-CNRS (France); Quentin Le Trequesser, Institut de Chimie de la Matière Condensée de Bordeaux-CNRS (France); Sonia L. C. Pinho, Institut de Chimie de la Matière Condensée de Bordeaux,-CNRS (France) and CICECO, Univ. of Aveiro (Portugal); Emeline Ribot, Ctr. National de la Recherche Scientifique (France); Pierre Voisin, Ctr. de Resonance Magnetique des Systemes Biologiques-CNRS (France); João Rocha, Univ. de Aveiro (Portugal); Herve Seznec, CENBG-CNRS (France)

This talk will stress on metal oxide nanoparticles (NPs) and two main aspects: their use as multifunctionnal contrast agents (CAs) and the quantification of their potential nanotoxicity at the sub-cellular level.

These multifunctionnal CAs can provide a multiple targeting and vizualisation of organs/cells with both detectable changes in MR signal intensities of the target and more classical fluorescent signals. We present an overview of our recent studies regarding the development of chemically modified biocompatible nanoparticles bearing gadolinium and europium chelates as MRI and fluorescent probes for targeted imaging with an example of their use in gene therapy applications. We will also present preliminary results on the introduction of a superparamagnetic oxide in the silica and study the interactions in between the shell and the core with respect to the silica shell thickness.

The second aspect deals with detection and quantification of NPs in tissues as well as deciphering of toxicity mechanisms. We illustrate the study of NPs subcellular distribution using ion beam analyses (IBA) showing our result regarding the interactions of functional NPs with Primary Human Foreskin Keratinocytes (PHFK). Cellular uptake, cytotoxicity, subcellular distribution, and quantification of NPs in tissues as well as origin of toxicity mechanisms are discussed here. The detection, quantification of internalized TiO2-NPs and intracellular elements using IBA (STIM and PIXE), and contribution to the study of nanotoxicity are presented. As an illustration of the methodology, we demonstrate, at the single cell, how NPs cellular uptake leads to in vitro intracellular calcium homeostasis alterations associated with early cell differentiation induction.

8955-23, Session 5

DNA templated optical antennas for surface-enhanced spectroscopy and biochemical sensing *(Invited Paper)*

Sebastien Bidault, Institut Langevin-CNRS (France)

By enhancing light matter-interactions in nanoscale volumes, gold nanostructures are particularly suited to design novel sensors and optical contrast agents. Furthermore, their robust surface chemistry and low oxidation facilitates their functionalization with various biomolecules. We demonstrated that dimers of gold nanoparticles (AuNPs) as large as 40 nm in diameter can be linked controllably by a single DNA strand (M. P. Busson et al, Nano Lett. 11, 5060, 2011) and feature significant scattering cross-sections. By introducing a single fluorescent molecule on the DNA linker, we can design new hybrid metal-organic chromophores with excitation cross-sections and decay rates enhanced by more than one order of magnitude with respect to isolated organic molecules (M. P. Busson et al, Angew. Chem. Int. Ed. 51, 11083, 2012) and that behave as single quantum emitters with high temporal coherence (M. P. Busson et al, Nat. Commun. 3, 962, 2012). We will discuss how the use of 60 nm and 80 nm AuNPs allows us to design bright emitters with picosecond lifetimes, corresponding to single photon sources with unprecedented dipolar transition moments.

The morphology of DNA templated AuNP dimers can be very sensitive to specific biochemical analytes. We recently showed that the interparticle distance in stem-loop containing AuNP dimers can be multiplied by 3 when hybridizing a specific target DNA single strand (L. Lermusiaux et al, ACS Nano 6, 10992, 2012). Recent results indicate how this topological shift can be translated into a macroscopic optical signal in order to design biochemical sensors at the single molecule limit.

8955-24, Session 5

Combining ligand design and photo-ligation to provide optimal quantum dot-bioconjugates for sensing and imaging *(Invited Paper)*

Naiqian Zhan, Goutam Palui, Malak Safi, Hedi Mattoussi, Florida State Univ. (United States)

We describe the synthesis of a series of compact ligands made of multi-coordinating (lipoic acid based) anchors, polyethylene glycol or zwitterion groups, and tunable terminal functions. We combine these ligands with a novel photo-ligation strategy to promote the transfer of QDs to polar and buffer media using such ligands, where in-situ reduction of the dithiolane, achieved under UV irradiation, is coupled with rapid ligand exchange on hydrophobic QDs. This strategy drastically improves previous transfer methods and is well adapted to the in-situ modification of QD surfaces with diverse functionalities. QDs with long term colloidal stability at sub-nanomolar concentrations under a range of biologically relevant conditions have been prepared and tested. This bodes well for in vivo imaging where very small concentrations are used. We also found that QDs stabilized with zwitterionc ligands are fully compatible with metal-histidine driven self-assembly with control the QD-protein conjugate valence. We will describe the ligand design, characterization, phase transfer, conjugate self-assembly and use for sensing ad imaging.

8955-25, Session 6

Interactions of gold nanoparticles with biological structures *(Invited Paper)*

Rute F. Fernandes, Neil Smyth, Antonios G. Kanaras, Univ. of Southampton (United Kingdom)

Understanding the interactions of colloidal nanoparticles with biological systems such as cells and more complex structures (i.e. skin) is essential for the development of new drug delivery methods, sensing and therapy. This presentation discuss how various physicochemical characteristics of nanoparticles influence their interactions with biological systems. In more detail, we will present our recent studies on how the charge and morphology of colloidal gold nanoparticles influence their penetration through various types of skins. Technical insights into the sample preparation will be also discussed.

8955-26, Session 6

Plasmonics with silver nanowires *(Invited Paper)*

Sebastian Mackowski, Nicolaus Copernicus Univ. (Poland)

Silver nanowires are among the most interesting plasmonic nanostructures. They not only participate in metal – enhanced fluorescence of located nearby emitters, but can also be used as nanoscale conductors or energy waveguides. In the presentation I will cover several aspects of research carried out in the Optics of Hybrid Nanostructures Group at the NCU in Torun. One of the most important results concerns strong, wavelength dependent fluorescence enhancement obse4rved for natural photosynthetic complexes coupled to silver nanowires. We have investigated the influence of particular geometry of a nanostructure upon the enhancement factors and find that the enhancement is a combination of absorption and emission rate increase in the photosynthetic complexes. Particularly interesting are results obtained for photosynthetic complexes bioconjugated with the nanowires, as in this way we achieve the ultimate control of the morphology of the structure. Another aspect of using silver nanowires in hybrid nanostructures concerns coupling polymers considered building blocks of organic solar cells with plasmon excitations in the nanowires. In a properly designed system, we obtained two-fold increase of the P3HT absorption. Finally, I will describe recent observations carried out on rare earth-doped nanocrystals placed in a proximity to the silver nanowires. As these systems feature reasonably efficient up-conversion, we demonstrate that this process can also be enhanced by the presence of the nanowires. All in all, the experimental results obtained for the nanostructures composed of silver nanowires, demonstrate excellent properties of these plasmonic structures, as well as unique versatility of possible applications.

8955-27, Session 6

The use of real-time optical feedback to improve outcomes

Isidro B. Magaña, Pratik Adhikari, Raghuvara B. Yendluri, Louisiana Tech Univ. (United States); Glenn P. Goodrich, Jon A. Schwartz, Nanospectra Biosciences, Inc. (United States); Patrick D. O'Neal, Louisiana Tech Univ. (United States)

More than a decade into the development of gold nanoparticles, with multiple clinical trials underway, ongoing pre-clinical research continues towards better understanding in vivo interactions with the goal of treatment optimization through improved best practices. In an effort to collect information for healthcare providers enabling informed decisions in a relevant time frame, instrumentation for real-time plasma concentration (multi-wavelength photoplethysmography) and protocols for rapid elemental analysis (energy dispersive X-Ray fluorescence) of biopsied tumor tissue have been developed in a murine model. An initial analysis, designed to demonstrate the robust nature and utility of the techniques, revealed that area under the bioavailability curve (AUC) alone does not currently inform tumor accumulation with a high degree of accuracy (R2=0.56), marginally better than injected dose (R2=0.46). This finding suggests that the control of additional experimental and physiological variables may yield more predictable tumor accumulation. Subject core temperature, blood pressure, and tumor perfusion are evaluated relative to particle uptake in a murine tumor model. New research efforts are also focused on adjuvant therapies which are employed to modify circulation parameters, including the AUC, of nanorods and gold nanoshells. Preliminary studies demonstrated a greater than 300% increase in average AUC through the use of a reticuloendothelial blockade agent versus control groups. Given a better understanding of the relative importance of the physiological factors which impact rates of tumor accumulation, a set of experimental best practices is presented.

8955-28, Session 6

Photoluminescence quantum yield of CdSe-ZnS/CdS/ZnS core-multishell quantum dots approaches 100% due to enhancement of charge carrier confinement

Pavel S. Samokhvalov, Pavel Linkov, National Research Nuclear Univ. MEPhI (Russian Federation); Jean Michel, Michael Molinari, Univ de Reims Champagne-Ardenne (France); Igor R. Nabiev, Univ. de Reims Champagne-Ardenne (France) and National Research Nuclear Univ. "MEPhI" (Russian Federation)

Modern nanotechnological applications of quantum dots (QDs) to biosensing and optoelectronics require the highest photoluminescence quantum yields, smallest FWHM, and strong protection of the luminescent nanoparticle from the environment. Usually, a high-band-gap shell is grown over the QD core to trap charge carriers in it and improve the photoluminescence emission outcome. A ZnS shell is commonly used with the CdSe cores because of its large band gap and environmental friendliness. The photoluminescence quantum yield is known to initially rise and then gradually fade with increasing thickness of the ZnS shell. On the other hand, a sufficiently thick shell efficiently protects the QD core and can reduce QD-photoluminescence blinking.

Here, we propose an approach ensuring strong charge carrier confinement in photoluminescent cores of CdSe-ZnS/CdS/ZnS core-multishell QDs. Unlike CdSe/CdS/ZnS or alloyed-shell QDs, where CdS serves to reduce the lattice strain at the core-shell interface, the multishell QDs contain a CdS interlayer in the ZnS shell introduced to break its continuity. This allows combining the advantages of the maximal potential barrier created by a single monolayer of the internal ZnS shell with excellent protection of the core and suitability of the external ZnS shell layer to QD-surface modification protocols generally used for QD solubilization and functionalization with capture molecules.

Using an adapted SILAR procedure, we have synthesized an extended series of CdSe-based core-shell QDs with various shell-layer combinations. We have found that CdSe-ZnS/CdS/ZnS core-multishell QDs have a highest photoluminescence quantum yield (approaching 100%) and a smallest FWHM compared with the CdSe/ZnS and CdSe/CdS/ZnS QDs.

8955-29, Session 7

Plasmonic biodegradable gold nanoclusters with high NIR-absorbance for biomedical imaging

Robert Stover, Avinash Murthy, Sai Gourisankar, Golay Nie, Miguel Martinez, The Univ. of Texas at Austin (United States); Thomas Truskett, University of Texas at Austin (United States); Konstantin V. Sokolov, Keith P. Johnston, The Univ. of Texas at Austin (United States)

Gold plasmonic nanoparticles are receiving attention for a variety of types of NIR optical biomedical imaging including photoacoustic imaging. Herein we present a novel method to assemble equilibrium gold nanoclusters from 5 nm primary gold nanospheres, which exhibit high near-infrared (NIR) absorbance and subsequently fully dissociate back to primary particles, which has the potential to enable renal clearance. The nanoparticle assembly is manipulated via controlling colloidal interactions, specifically electrostatic repulsion and depletion attraction. The charge on the primary ~5 nm gold nanospheres is tailored via place exchange reactions with a variety of biocompatible ligands such as citrate, lysine and cysteine. The primary particles form clusters upon addition of a biodegradable polymer, PLA(1k)-b-PEG(10k)-b-PLA(1k), followed by controlled solvent evaporation. The cluster size may be tuned from 20-40 nm in diameter by manipulating the gold and polymer

concentrations along with the solvent evaporation extent. In some cases, salt is also added to increase the NIR absorbance and reduce the nanocluster size by reducing polymer adsorption. The adsorption of the polymer onto the Au surfaces effectively quenches the nanoclusters. High NIR absorption at 1064 nm facilities photoacoustic imaging, even for the small cluster sizes. In response to acidic cellular pH environments, the polymer degrades and the clusters dissociate back to primary particle on the order of 5 nm, which are small enough for renal clearance.

8955-30, Session 7

Evaluation of quantum dot-based concentric FRET configurations with a fluorescent dye and dark quencher for multiplexed bioanalyses *(Invited Paper)*

Erin M. Conroy, W. Russ Algar, The Univ. of British Columbia (Canada)

The unique optical properties of semiconductor quantum dots (QDs) combined with Foerster resonance energy transfer (FRET) have attracted considerable interest for assay and biosensor development. While many of these developments have required sophisticated optical readout instrumentation, there is a need for simple, low-cost readout systems for point-of-care diagnostic applications. In this presentation, recent efforts by our group to develop and test such systems on paper platforms will be described. Methods have been developed for modifying cellulose paper substrates with QD donors and acceptor dye-labeled biomolecule probes. Interestingly, energy transfer rates between the QDs and dye molecules are enhanced four-fold within the paper matrix resulting in enhanced acceptor emission and more sensitive detection. The QD-FRET system can be interrogated using a low-power violet light emitting diode (LED) or a hand-held black light. QD and FRET-sensitized dye photoluminescence (PL) can be measured using portable, low-cost CMOS devices such as a smartphone camera or webcam. The built-in color filters in the cameras permit resolved detection of QD and dye PL in FRET configurations with fluorescent acceptors, or detection of three distinct colors of QD in FRET configurations with dark quenchers. We show that the activity associated with nanomolar levels of protease can be detected using the above systems, including multiplexed detection of up to three proteases and pro-enzyme activation assays. Our progress in designing assays that provide semi-quantitative readout of proteolytic activity by eye will also be discussed. The bright, narrow PL of QDs is very promising for developing point-of-care assays.

8955-31, Session 7

Biophotonic logic devices that use multiple fluorescent (Förster) resonance energy transfer relays from a single quantum dot bioconjugate *(Invited Paper)*

Jonathan C. Claussen, U.S. Naval Research Lab. (United States) and George Mason Univ. (United States); W. Russ Algar, The Univ. of British Columbia (Canada); Niko Hildebrandt, Institut d'Électronique Fondamentale (France); Kimihiro Susumu, U.S. Naval Research Lab. (United States) and Sotera Defense Solutions (United States); Mario G. Ancona, Igor L. Medintz, U.S. Naval Research Lab. (United States)

Biophotonic logic devices (BLDs) hold tremendous promise for a wide variety of applications including sensing, medical diagnostics, theranostics, and nanorobotics. These devices typically utilize chemical/biochemical inputs that are transduced to electrochemical or photonic output signals. Such nonhomogeneous standard Input/Output (I/O) signals greatly limit circuit complexity as logic gate concatenation is rendered nearly impossible. However, we demonstrate BLDs that use photonic integration with distinct dye-peptide inputs in conjunction with quantum dots (QD) to produce photonic output signals that create logic gates and complex logic circuits. These circuits operate through multiple Fluorescent (Förster) Resonance Energy Transfer (FRET) relays that very spatially and temporally. Recently we have shown that distinct photoluminescence (PL) spectra can be accessed from a single excitation wavelength with QD-fluorophore conjugates. By conjugating both long lifetime luminescent terbium(III) complexes (Tb) and fluorescent dyes (A647) to a single QD, we can create multiple FRET relays that change temporally as the QD acts as both an acceptor and donor at distinct time intervals. Likewise, distinct QD-dye FRET pairs with defined spatial separation can create a unique PL spectra from a single excitation. In this work we fabricate unique three luminophore systems (Tb-QD-A647) with distinct valences of Tb (Input A) and A647 (Input B) to a central QD to create multi-step FRET cascades that are capable of performing both combinatorial and sequential logic. Such sophisticated logic operations open the door to a wide range of applications including multiplexed in vivo biodetection and biosensing for disease diagnostics and treatment.

8955-32, Session 7

Direct detection of multiexciton dynamics in single colloidal quantum dots

Thomas S. Bischof, Moungi G. Bawendi, Massachusetts Institute of Technology (United States)

Multiexcitons define the fundamental limit of light generation in colloidal quantum dots (CQD). While these states are not yet thoroughly understood, they are involved in a variety of important phenomena such as carrier multiplication and photoluminescence blinking. Several techniques have been developed recently to unravel the details of these dynamics.

One such technique relates the extent of antibunching of emission from single colloidal quantum dots to biexciton emission statistics. As with other single-molecule methods, this permits us to study the heterogeneity of dynamics among ostensibly identical molecules, and provides a direct window into manipulation of their fundamental physical properties. Various researchers have used this method to demonstrate that the thermal, chemical, and electronic environments of quantum dots can have profound effects on multiexciton statistics.

Here we present an extension of this technique to higher orders of correlation and antibunching. The method permits direct observation of the roles of multiexcitons in photoluminescence blinking, and we find that some CdSe-based dots exhibit blinking which can be explained by exciton blinking--without corresponding biexciton quenching. We also combine the method with fluorescence correlation spectroscopy (FCS) to enable high-throughput analysis of multiexciton quantum yield in batches of quantum dots.

This technique also provides a direct method for individually resolving the dynamics of multiexciton emission, and we report the pure lifetimes of the exciton through the quadraexciton.

8955-33, Session 7

Interferometric measurements of cavitation bubbles around laser irradiated gold nanoparticles

Florian Rudnitzki, Norbert Linz, Sebastian Freidank, Alfred Vogel, Gereon Hüttmann, Univ. zu Lübeck (Germany)

Due to their unique optical properties gold nanoparticles are suitable agents for targeted cell elimination or manipulation in flow cytometry. Specific targeting is established by antibodies. Irradiation of the accumulated spherical or cylindrical gold nanoparticles at certain

wavelengths induce different processes, which can lead to destruction of biological structures. Photothermal induced cavitation bubbles around the gold nanoparticles were believed to be the main responsible effects for cell permeabilisation and cell killing.

The dynamics of cavitation bubbles in dielectrics has been investigated extensively, while the dynamics of cavitation bubbles around plasmonic particles remains in parts still unknown. We developed an interferometry setup, which allows measurement of cavitation bubbles around gold nanoparticles with high temporal and spatial resolution. In contrast to transmission setups used so far, the measurements in reflection give information about the velocity of the bubble wall in the expansion and collapsing phases of the laser induced bubble.

More important our setup allows measurements at non-transmitting or strongly scattering interfaces such as cell membranes. With the setup, the nucleation of cavitation bubbles can be correlated with the applied laser energy, and the irradiation wavelength as well as the optical properties of the used nanoparticles. Thus, modeling of the optical properties and temperature distribution in and around gold particles can be refined.

8955-70, Session 7

Droplet-based high-throughput screening assays using quantum dot barcode labels *(Invited Paper)*

Ralph A. Sperling, Institut für Mikrotechnik Mainz (Germany) and Harvard Univ. (United States); Adam R. Abate, Harvard Univ. (United States) and Univ. of California, San Francisco (United States); David A. Weitz, Harvard Univ. (United States)

Microfluidic droplets can be seen as the miniaturized analog of reaction tubes: Small isolated compartments of a liquid, typically aqueous, separated by an inert outer phase, e.g. fluorinated oil. The droplets can be generated in a very controlled fashion at at a very high rate in simple microfluidic devices. Reagents can be added to droplets, the droplets can be manipulated on-chip or stored outside and reinjected at a later point. The contents of droplets can be optically detected by fluorescence. Taken together, generation, manipulation and detection of microfluidic droplets present a new approach to screening assays, where the reduction in sample size from microliters to picoliters offers a reduction of reagent cost of several orders of magnitudes, as it does in regard to processing time. However, interfacing microfluidic devices, i.e. the transition from macro to micro while keeping fluid volumes minimum, is still a challenge. We have developed a method for the parallel encapsulation of samples, allowing for the efficient generation of droplet libraries. By that, a large number of reagents can be encapsulated into droplets and stored for later use. Individual members of the library can labeled prior to encapsulation by fluorescent barcode labels, e.g. generated with different concentrations of flurescenct labels such as quantum dots. For a screening assay, the samples to be screened are injected into each of the droplets of the pre-formed library, while all reactions are kept isolated inside the individual drops. The outcome of the reaction is read out optically for each individual droplet, while the library member is identified by its fluorescent barcode label. By this, the ‘expensive’ droplet library that contains all reagents to be screened, has to be formed only once and can be used for many experiments. As eventually for each screening reaction only sub-nanoliter droplets are needed, reagent cost will be greatly reduced. Possible applications include genotyping, sequencing, or drug screening.

8955-39, Session PSun

In vitro and in vivo antitumor efficacy of curcumol nanosuspension against H22 tumor

Zhiping Wang, Guangdong Pharmaceutical Univ. (China); Junbiao Wu, Guangzhou Univ. of Chinese Medicine (China); Qun Zhou, Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, Tongji School of Pharma (China); Tongsheng Chen, South China Normal 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 resistence of the advanced hepatocarcinoma to chemotherapy. Curcumol (Cur) is a novel compound extracted from Chinese curcumae rhizoma. It exhibits various anticancer activities and can be used to treat hepatocarcinoma. However, the compound’s low solubility hinders its development. Recently nanosuspension has been developed as one of the most promising formulations for poorly water soluble drugs. In this report, we evaluate both the in vitro and in vivo antitumor activity of a curcumol nanosuspension (Cur-N) relative to efficacy of bulk Cur delivery. Cur-N with a particle size of 231.2 ± 7.2 nm and a zeta potential of ?27.3 ± 0.6 mV was prepared by the high-pressure homogenization (HPH) technique. The in vitro cytotoxicity of Cur-N against H22 cells was evaluated by CCK-8 assay. The in vivo antitumor activity was observed in H22 tumor bearing mice. CCK-8 assay showed that Cur-N effectively inhibited the proliferation of H22 cells. In vivo studies Cur-N also showed higher antitumor efficacy as measured by reduced tumor volume and tumor weight, as well as lower toxicity in H22 solid tumor bearing mice compared to free Cur at the same concentration. These results suggest that the delivery of Cur as a nanosuspension is a promising approach for treating tumors.

8955-59, Session PSun

Iron oxide nanoparticles in different modifications for antimicrobial phototherapy

Elena S. Tuchina, N.G. Chernyshevsky Saratov State Univ. (Russian Federation); Kristina V. Kozina, Nikita A. Shelest, NG Chernyshevsky Saratov State Univ (Russian Federation); Vjacheslav I. Kochubey, Valery V. Tuchin, N.G. Chernyshevsky Saratov State Univ. (Russian Federation)

The main goal was to study the sensitivity of two strains of Staphylococcus aureus to action of LED blue (405 nm) light and Fe2O3, Fe3O4 nanoparticles and their conjugates with TiO2 core.

Two strains of Staphylococcus aureus were used: meticillin-sensitive and meticillin-resistance.

As LED blue light source with spectrum maxima at 405 nm (70 mW/cm2) was taken. The light exposure was ranged from 5 to 30 min. Nanoparticles with average size about 15-25 nm in different concentrationswere used.

It was shown that irradiation with blue light caused a decrease in number of microorganisms treated with nanoparticles of 20 - 95%.

8955-60, Session PSun

NaGdF4 nanocrystals as multifunctional diagnostic agents

Mateusz Banski, Bartlomiej Sojka, Agnieszka Noculak, Wroclaw Univ. of Technology (Poland); Jana Tulinska, Slovak Medical Univ. (Slovakia); Artur P. Podhorodecki, Jan Misiewicz, Wroclaw Univ. of Technology (Poland)

NaGdF4 nanocrystals (NCs) doped with lanthanide ions are promising nanomarkers which overcome problems of traditional fluorophores such as organic dyes and fluorescent proteins, which suffer from a rapid photobleaching, spectral cross-talking, blinking and limited brightness. Simultaneously, NaGdF4 NCs are much less toxic than cadmium based quantum dots. For application as bio-markers, the high quality

nanocrystals smaller than 10 nm are favorable. Preparation of hexagonal NaYF4 matrix is also important, as the luminescence comprising excited ion-ion interactions is an order of magnitude less effective in cubic NCs, compared to hexagonal ones.

Here, we present the effective method of sub-5 nm NaGdF4 NCs synthesis. We examine the influence of a reduced size of nanocrystals on their optical properties and photostability. Then, we exploit the co-doping of Ln3+ ions into fluoride nanocrystals as a promising method of NCs functionalization. We confirm that Gd3+ ions play a role of efficient sensitizers, which transfer absorbed energy to e.g. Tb3+ ions. Further Tb3+ and Eu3+ co-doping in NCs results in a multicolor emission, which is due to phonon-assisted energy transfer. At the same time, it enables the application of NaGdF4:Tb3+, Eu3+ NCs as optical nanothermometers. We also demonstrate the magnetic properties of our NCs in order to examine their potential application as dual-mode markers. Finally, we investigate the immunotoxic effect of hydrophobic fluoride nanocrystals to lymphocytes and phagocytes. Combination of all the functionalities in a single nanoparticle clearly show the potential of NaGdF4 NCs as multifunctional diagnostic agents.

8955-61, Session PSun

CdTe quantum dots conjugated to monoclonal antibodies for investigation of A and B antigens on red blood cells membrane

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The ABO is considered the most important blood system for transfusion, transplants and criminal forensics purposes. Time consuming molecular biology techniques are usually employed to investigate ABO groups and subgroups. Therefore, new methods using quantum dots (QDs) as fluorescent probes conjugated to organic molecules to label specific biological structures have been proposed. However, an effective bioconjugation can still be considered a challenge, because it has to preserve the QDs fluorescence and the biomolecules functionality. Therefore, by overcoming this challenge, QDs could be used to elucidate several immunohematology features. In this work, we applied CdTe QDs covalently binded to anti-A or anti-B monoclonal antibodies to investigate red blood cells (RBCs) antigens of A1, B1, A1B1 and A subgroups with flow cytometry. We evaluated the bioconjugation by fluorescence correlation spectroscopy (FCS) corroborated by electrophoresis assays. The correlation curves, and QD sizes for bare and conjugated QDs extracted from them, demonstrated the success of our QDs conjugation to the antibodies. QDs-(anti-A) labeled around 97% of A1 RBCs, 95% of A1B1 and it was able to distinguish some A subgroups both by profile and labeling efficiency (A2 – 68%; A3 –11% and Ax – 5%). Furthermore, QDs-(anti-B) effectively labeled B1 and A1B1 RBCs (95% and 80%). We demonstrated, therefore, that QDs and their bioconjugates can be applied as a less laborious, low cost, quantitative and complementary tool for the comprehension of the erythrocyte biology.

8955-62, Session PSun

Quantum dots conjugated to lectins as nanoprobes for cell surface carbohydrates detection in human mammary glands

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The recognition of glycans as mediators of important biological processes has stimulated the interest in researches related to glycobiology. Studies of cell surface carbohydrates have revealed that malignant transformation is associated with a variety of altered cell glycosylation patterns. Identifying these changes in glycoconjugates at an early stage of the tumor development may offer the possibility to understand their role in disease development as well as monitoring disease progression. In this context, conjugates composed by lectins and fluorescent labels are promising molecular probes to investigate changes in the expression of glycoproteins. The aim of this study was to evaluate the expression of glycoconjugates in normal human breast tissue, benign (fibroadenoma) and malignantly transformed tissues (invasive ductal carcinoma - IDC). For this, it was applied CdTe quantum dots, synthesized and functionalized/stabilized with mercaptosuccinic acid (MSA), conjugated to Concanavalin A (Con A) and Ulex europaeus agglutinin I (UEA-I) lectins to detect, respectively, ?-D-glucose/mannose and L-fucose residues. The conjugates were evaluated by absorption and emission spectroscopy, hemagglutination activity tests and carbohydrates inhibition assays. These tests showed that the QDs-lectin conjugates remained functional, keeping its fluorescent properties and the carbohydrate recognition ability. Fluorescence images showed that different regions of breast tissues expressed different and particular types of carbohydrates. While the stroma is preferentially and intensely stained by QDs-(Con A), clearly QDs-(UEA-I) preferred to label ductal cells. The results indicate that QDs-lectins conjugates can be used as molecular probes and thus help to elucidate pathological processes.

8955-63, Session PSun

Photoirradiation study of gold nanospheres and rods in Vero and Hela cell lines

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Photoirradiation effect of gold nanospheres and rods in conjunction with Green and red light were found to be appreciable. In this present work, the concentration of nanomaterial and light dose were optimized. Gold nanospheres were synthesized by reduction technique using Sodium Borohydrate as reducing agent and Trisodium Citrate as capping agent. Au nanorods synthesized using reduction techniques with Cetyl Trimethyl Ammonium Bromide(CTAB) and benzyl dimethyl hexadecyl ammonium chloride (BDAC) polymers observed to have absorption in the region of 680-900nm. UV-Vis absorption and Transmission Electron Microscopy studies confirmed the size of nanoparticles. To Vero and Hela cell lines, Gold nanospheres of size 30nm and green light source of 530nm wavelength with power 30mW were applied. Similarly, Nanorods were applied and irradiated with 680nm wavelength light source with intensity of 45mW. The cell death rate was slightly higher for spheres when compare with rods. The cell death rate was higher for Hela cells when compare to Vero cells. Post irradiation effect for 24hrs, 48hrs confirms cell proliferation in normal rate in viable cells. The morphological changes in irradiated spot leads to apoptotoic cell death was confirmed with microscopic imaging. The LD50 value was also calculated.

Key words: Photoirradiation, Gold Nanospheres, Gold Nanorods, Apoptosis

8955-64, Session PSun

Point-of-care test for protein content analysis in punctuate liquids based on gold nanoparticles

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Increased protein concentrations in liquor cerebrospinalis samples are an indicator of several neurological deseases. There is a need for a near patient test in the emergency medicine. Based on gold nanoparticles (GNP) a simple and fast protein test system is developed using a test liquor made of albumin (main constituent of real liquor) and phosphate buffered saline. After addition of this test liquor to a suspension of GNPs it is found that albumin concentrations (µg/ml range) trigger an agglomeration of the nanoparticles. The agglomeration effects a colour change from red to violet/blue in the relevant concentration range.

8955-65, Session PSun

Stimuli-induced, directed actuation of micro/nanoparticles from a multiblock copolyester urethane

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Polymer based micro- and nanoparticles are of practical relevance as injectable carriers systems for biomedical applications. Based on recent discussions on the role of particle shape for the biorecognition and biodistribution of micro-/nanoparticles, the capability of particles to be altered in their shape by a stimuli-induced, spatially directed actuation should be explored. Micro- and nanoparticles were prepared from a multiblock copolyester urethane based on poly(?-caprolactone) and poly(?-pentadecalactone) segments, which exhibited a semi-crystalline morphology with a physical network structure. By facilitating the concept of shape-memory polymers that can store mechanical stress in a polymer network, particles of a non-spherical temporary shape were prepared by a thermomechanical programming process. These prolate ellipsoids could switch back to their permanent spherical shape by application of heat (e.g. 60 °C) as a stimulus. For matrices in the low nanometer size range, it can be expected that there are size limitations for the shape-memory functionality of such particles due to a decreasing degree of crystallinity of the polymer [1]. The interaction of either spherical or non-spherical multiblock-copolymer particles with macrophages as phagocytic cells has also been studied. Externally induced switching at physiological temperature could lead to carriers that can be subject to an on-demand biorecognition.

[1] C. Wischke, M. Schossig, A. Lendlein. Shape-Memory Effect of Micro-/Nanoparticles from Thermoplastic Multiblock Copolymers. Small, DOI: 10.1002/smll.201202213.

8955-66, Session PSun

Thin film mesoscale organization of nanoparticles by using biomolecular peptide tools

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Highly ordered organization of nanoparticles on the surface of biological self-assembled molecules has opened a new era for the fabrication of novel electronic materials. Templating semiconductor nanoparticles' growth on the surface of self-assembled proteins and peptides is a promising avenue over the limitations that top-down techniques may impose on the fabrication of nanoscale devices. Here we report the development of two-dimensional ordered structures of preformed TOPO (trioctylphosphine oxide) capped CdSe@ZnS core-shell quantum dots (Qdots, with green and red fluorescence emission) on self-assembled peptide fibrils. An amphiphilic peptide was employed both as ligand-exchange element (via its cysteine residues) and as a structural scaffold for the ordering of Qdots at the water-chloroform interface. With the aid of transmission electron microscopy we discuss the possible topological arrangement of the Qdots as imposed by the peptide fibril film. The physical properties of the final assembled structures differ from the corresponding individual semiconducting nanocrystals. The assembly of the Qdots in close proximity is claimed to induce a characteristic red-shifted spectral response, which is discussed on the basis of long-range electronic energy transfer. The exciting technological prospects of the films entailing coupled quantum dots are illustrated by their pronounced photoluminescence emission.

8955-67, Session PSun

Shielding of quantum dots using diblock copolymers: Implementing copper catalyzed click chemistry to fluorescent quantum dots

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We describe the design and optimization of two surface functionalization strategies that can provide hydrophilic and functional QDs ideally suited for bio-functionalization using hydrazone ligation, or copper-catalyzed "click" reaction (azid alkyne Husigen cyloaddition).

In the first design we use a photoligation strategy to promote the transfer of QDs to buffer media that circumvents the need of chemical reduction of lipoic acid. This approach promotes the capping of QD with aldehyde-modified lipoic acid ligand (via a multidentate anchoring) while maintaining the group's chemical integrity. The resulting QDs are readily reactive with amines, hydrazines and oxylamines groups, which can be used to conjugate the nanocrystals with biomolecules under physiological conditions. Using suitable nucleophiles, namely hydrazinopyridine a new chromophore is formed during this reaction, providing an optical signature that can be monitored by UV spectroscopy. Furthermore, this chromophoric signature can be used to quantify the rate of reaction, and ultimately an estimate for the number of ligands per nanocrystal.

In the second design, copper catalyzed cycloaddition (click), applied to QDs that maintain their fluorescence properties was carried our using a micellar encapsulation with poly(isoprene-block-ethylene oxide) block-copolymers. Due to the hydrophobic character of the block-copolymer this approach provides a shielding layer surrounding the particles, which prevents metal ions from reaching the QD surface and maintain their fluorescence properties during and after reaction.

8955-68, Session PSun

Nanotech cream for cutaneous treatment: a toxicology and permeability study

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Colloidal iron oxide nanoparticles (NPs) can be designed in countless different ways and their clinical applications in drug delivery and diagnosis are seriously being considered. These nanoparticles present several advantages: superparamagnetic behavior and amphiphilic polymer surface coating introduce their well-known biocompatibility, while the presence of functional groups on the surface allows for the attachment of bioactive molecules. In view of development of broadly applicable pharmacological formulations for local and systemic diseases, the cutaneous treatment still represents the most investigated way between available administration routes. The goal of this work is to evaluate NPs permeability through the human skin tested in two different formulations with specific experimental set-up in vitro and in vivo: a stable suspension of iron oxide nanoparticles and the same nanoparticles incorporated in a base-cream. For future applications it was also necessary to study potential toxic effects of nanoparticles in human fibroblastic cells. Iron oxide nanoparticles labeled with fluoresceine were formulated in an Essex cream for its moisturizing properties that should promote hydrophilic-coated nanoparticle adsorption. Using progressive dilution, nanoparticles were included up to a concentration of 8 ?gNPs/mg cream. Planning MTT assay and cell count, we demonstrated a non-toxic behavior for nanoparticles concentrations of 5, 10, 20, 50, 100 ?g/mL although at the maximum concentration tested (200 ?g/mL NPs exhibited some toxic effects. For in vitro analysis, Franz diffusion cell system was employed, that showed the nanoparticles penetration through all layers of human skin in both types of formulations. For in vivo analysis, the treatment with nanoparticles formulated in cream was evaluated on 1 cm2 of mice skin. The data from flow cytometry indicated nanoparticles uptake from skin macrophages and dendritic cells.

8955-69, Session PSun

Exploring the use of colloidal gold for nonthermal photodynamic cancer therapies

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Hyperthermia cancer therapy based on plasmonic heating of gold nanoparticles or nanorods has been suggested as an alternative to the use of superparamagnetic nanoparticles. [1] However, it is unclear to what extent thermal effects are the dominant cause of cell damage when intracellular gold nanoparticles are exposed to laser light. Here we demonstrate that local damage to intracellular structures and even cell death is observed in the presence of gold nanoparticles at low laser power intensities where heating is negligible.[2] This indicates the presence of a photochemical process responsible for cell death.

HeLa cells, a human fibroblast cell line, internalise 13nm gold nanoparticles, and when exposed to laser irradiation under plasmon resonance conditions, the cells die. The cells do not die during or immediately after exposure to the laser, instead, there is a delay between exposure and cell death dependent on the irradiation time and intensity. Comparing this to HeLa cells heated in a water bath confirms that the method of killing is not thermal.

At present, the photochemical process that induces cell death is being investigated by determining whether cell death is apoptotic or necrotic and by the detection of cytotoxic products of the photochemical process, such as singlet oxygen or free radicals.

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2. Krpetic, Z., et al., Inflicting Controlled Nonthermal Damage to Subcellular Structures by Laser-Activated Gold Nanoparticles. Nano Letters, 2010. 10(11): p. 4549-4554.

8955-72, Session PSun

PEGylated magnetic graphene oxide for dual targeted delivery of doxorubicin and photothermal therapy

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Magnetic graphene oxide (MGO) was prepared by chemical co-precipitation of Fe3O4 magnetic nanoparticles on GO nano-platelets and subject to further modification by polyethylene glycol (PEG) and grafting with an epidermal growth factor receptor (EGFR) monoclonal antibody (cetuximab, CET). Since EGFR is highly expressed on the surface of certain tumor cells, MGO-PEG-CET could be used for dual (magnetic and ligand-mediated) targeted delivery of an anticancer drug (doxorubicin, DOX) after loading the drug through π-π stacking interactions with GO. Drug loading experiments indicated under optimum condition, up to 325.9% (w/w) of DOX could be loaded on MGO-CET. Intracellular uptake studies using quantum-dots labeled nanocarrier and fluorescence imaging indicated uptakes of MGO-CET by EGFR-expressed CT-26 murine colorectal cells was more efficient than MGO and inhibited by excess CET, confirming ligand-mediated endocytosis of the nanocarrier. Cell cytotoxicity studies using MTT assays also showed the IC50 of MGO-CET/DOX (1.48 ?g/mL) is lower than that of MGO/DOX (2.64 ?g/mL). After combination with thermal therapy using near-infrared laser light exposure for 5 min at 2.5 W/cm2, the IC50 could be further reduced to 1.17 ?g/mL. In vivo anti-tumor studies with subcutaneously implanted CT-26 cells in BALB/c mice showed the relative (to day 0) tumor volumes at day 14 were 0.42, 5.80, 9.53, 10.14 and 12.13 for MGO-CET/DOX + magnet + laser, MGO-CET/DOX + magnet, MGO-CET/DOX, free DOX and normal saline groups, respectively. Our results showed a 24–fold increase in the cancer therapeutic response for the combination of photothermal therapy and dual targeted delivery of DOX using PEGylated MGO.

8955-73, Session PSun

Quantum dots (QDs) restrain human cervical carcinoma HeLa cell proliferation through inhibition of the ROCK-c-Myc signaling

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Cancers often cause significant morbidity and even death to patients. To date, conventional therapies, such as chemotherapy, radiation and surgery, are often limited; meanwhile, novel anticancer therapeutics are urgently needed to improve clinical treatments. Rapid application of nanotechnology and nanomaterials represents a promising vista for the development of anti-cancer therapeutics. However, how to integrate the novel properties of nanotechnology and nanomaterials into cancer treatment warrants close investigation. In the current study, we report a novel finding about the inhibitory effect of CdSe quantum dots (QDs) on Rho-associated kinase (ROCK) activity in cervical carcinoma HeLa cells associated with the attenuation of the ROCK-c-Myc signaling. We mechanistically demonstrated that QD-conducted ROCK inhibition greatly diminished c-Myc protein stability due to reduced phosphorylation, and also suppressed

its activity in transcribing target genes (e.g. HSPC111). Thus, the

treatment of QDs greatly restrained HeLa cell growth by inducing cell cycle arrest at G1 phase due to the reduced ability of c-Myc in driving cell proliferation. Additionally, since HSPC111, one of the c-Myc targets, is involved in regulating cell growth through ribosomal biogenesis and assembly, the downregulation of HSPC111 could also contribute to diminished proliferation in HeLa cells upon QD treatment. These results together suggested that inhibition of ROCK activity or ROCK-mediated c-Myc signaling in tumor cells upon QD treatment might represent a promising strategy to restrain tumor progression for human cervical carcinoma.

8955-34, Session 8

Plasmonic-driven thermal sensing

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In the last decades, inorganic nanoparticles have been steadily gaining more attention from scientists from a wide variety of fields such as material science, engineering, physics or chemistry. The very different properties compared to that of the respective bulk, and thus intriguing characteristics of materials in the nanometre scale, have driven nanoscience to be the centre of many basic and applied research topics. Moreover, a wide variety of recently developed methodologies for their surface functionalization provide these materials with very specific properties such as analyte detection, for example detection of circulating cancer biomarkers.

In this work we describe a highly sensitive biosensor for the carcionoembryonic antigen (CEA) based on the use of gold nanoprisms derivatized with specific anti-CEA antibodies, nitrocellulose membranes and fax paper as thermosensitive support. Plasmonic heating properties of the gold nanoprisms were used as transducer signal for the detection of CEA. A dot-blot like assay has been developed using derivatized gold nanoprisms as detection unit. In a typical experiment, nitrocellulose membranes were functionalized with anti-CEA. In the presence of the analyte, anti-CEA derivatized nanoprisms will be retained on the nitrocellulose membranes. Irradiation with a NIR laser produced an intense signal in the thermosensitive support induced by the increase of temperature by the heating of nanoprisms after irradiation. The intensity of the signal is directly related with the analyte concentration. Real patient samples have been used, obtaining sensitivities up to attomolar range by simple eye detection.

8955-35, Session 8

Gold nanosponges: plasmonic properties of single-nanoporous gold nanoparticles *(Invited Paper)*

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Single nanoparticle spectroscopic studies of a novel nanoparticle morphology comprising nanoporous gold nanoparticles, so-called nanosponges, are presented. While usual colloidal nanoparticles possess rather moderate surface-area-to-volume ratios, the nanosponges exhibit high surface-area-to-volume ratios as well as plasmon resonances overlapping with the so called ‘tissue diagnostic window’. Combing the properties of plasmonic nanoparticles and nanoporous metallic materials, the nanosponges are predestined for bio applications. On the one side, the high surface-area-to-volume ratios are crucial for biosensing, SERS and fluorescence applications, as well as for electrochemistry and catalysis. On the other side, the spectral positions of their plasmon resonances render the nanosponges promising candidates for in vivo imaging and sensing application.

8955-36, Session 8

Small upconversion fluorescent nanoparticles for photoactivation *(Invited Paper)*

Yong Zhang, Muthu Kumara Gnanasammandhan Jayakumar, Akshaya Bansal, National Univ. of Singapore (Singapore)

Traditional fluorophores including fluorescent dyes/proteins and quantum dots (QDs) are based on ‘downconversion fluorescence’, emitting low energy fluorescence when excited by high energy light such as UV or short wavelength visible light. They have several drawbacks: photobleaching, autofluorescence, short tissue penetration depth and tissue photo-damage. Upconverting lanthanide doped nanocrystals (UCNs) emit detectable photons of higher energy upon excitation by near-infrared (NIR) light based on a process termed ‘upconversion’. UCNs show absolute photostability, negligible autofluorescence, high penetration depth and minimum photodamage to biological tissues. They can be used for ultrasensitive interference-free biodetection because most biomolecules do not have upconversion property. UCNs are also useful for photoactivation. Various biomolecules like DNA, RNA, proteins, drugs and ligands are being used for diverse therapeutic and experimental uses. One interesting strategy is to cage the molecules with a light-sensitive compound and deliver them. After delivery, the caged molecules will be activated specifically at the site of interest by shining a light source and uncaging the molecules, making it functional. But a major shortcoming of this efficient technology is that most of the caged compounds need UV light for uncaging. UV light cannot be used for in-vivo applications due to its toxicity and low penetration depth. Near-infrared (NIR) light can penetrate into tissues due to weak absorption. So, upconversion fluorescent nanoparticles are used as a nanotransducer for photoactivation of biomolecules. These nanoparticles absorb NIR light and convert it to UV/Visible light which can then be used to activate the photocaged biomolecules.

8955-37, Session 8

Large nonlinear and linear optical responses in a hybrid nano-biomaterial engineered from bacteriorhodopsin and semiconductor quantum dots

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The development of bio-inspired hybrid nanomaterials that can be made competitive with existing materials upon their integration in current technologies is one of the interesting challenges for modern science and technology. Protein-based photonic devices have been paid special attention because of the possibility to control the functional and optical properties of proteins through chemical modifications, genetic engineering, and specific spatial organization via self-assembly. Here, the membrane protein bacteriorhodopsin (bR), a single integral protein of purple membranes (PM) of Halobacterium salinarum, has attracted most attention.

Förster resonance energy transfer (FRET) between semiconductor quantum dots (QDs) and bR in its natural PM may be conrolled by tuning of the Förster radius, overlap integral between the donor emission spectrum and the acceptor absorption spectrum, and the distance between the donor (QD) and acceptor (bR-retinal). The observed energy transfer from QDs to bR corresponds to that predicted by a multiple-acceptor geometric model describing the FRET phenomenon for QDs

quasi-epitaxied on a crystalline lattice of bR trimers. Linking of QDs and bR via streptavidin–biotin linkers of different lengths improved FRET with an efficiency as high as 82%, substantially exceeding the values predicted by the classical FRET theory.

Additionally, QD-PM binding produces wavelength-dependent enormous enhancement of the nonlinear refractive index of hybrid material with the strongest effect in the region just below the absorption edge of both constituents of this hybrid material and in samples that show strongest FRET. An enhancements of up to 4000% can be achieved by controlled engineering of the hybrid structure. This new hybrid material with exceptional nonlinear properties will have numerous photonic and optoelectronic applications employing its photochromic, energy transfer, and conversion properties.

8955-38, Session 8

Noncytotoxic Mn-doped ZnSe/ZnS quantum dots for biomedical applications

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Quantum dots (QDs) emitting in the visible are of interest for many biomedical applications, including bioimaging, biosensing, drug targeting, and photodynamic therapy. However, a significant limitation is that QDs typically contain cadmium, which makes prospects for their FDA approval very unlikely. We have synthesized Cd-free Mn-doped ZnSe/ZnS QDs coated with peptides and conducted an ApoTox-Glo Triplex assay to determine their cytotoxic effects on macrophage cells. This particular assay provides information regarding cytotoxicity, viability, and induced apoptosis effects that the material under test may have on the cells. Markers for cell viability and cytotoxicity were simultaneously measured with fluorometric assay reagents. Induced apoptosis was measured from a luminogenic reagent that provided a luminescent signal proportional to caspase activity, a biomarker for apoptosis. The measurements were taken over four different QD concentrations: 0.25, 0.125, 0.0625, and 0.03125 μM, and over four different incubation times: 6, 12, 24, and 48 hours. After 6 hours of incubation, cell viability was not affected by the presence of the QDs. With the increase of incubation time to 12, 24, and 48 hours, cell viability increased beyond that of the untreated cells, suggesting an enhancement in viability due to the presence of the QDs.

8955-40, Session 9

Nanocapsules of perfluorooctyl bromide for theranostics: from formulation to in vivo targeting *(Invited Paper)*

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The need to detect cancer at its early stages, as well as, to deliver chemotherapy to targeted site motivates many researchers to build theranostic platforms which combine diagnostic and therapy. Among imaging modalities, ultrasonography and MRI are widely available, non invasive and complement each other. Both techniques often require the use of contrast agents.

We have developed nanocapsules of perfluorooctyl bromide as dual contrast agents for both imaging modalities. The soft, amorphous polymer shell provides echogenicity, while the high-density perfluorinated liquid core allows detection by 19 F MRI. We have used a shell of poly(lactide-co-glycolide) (PLGA) since this polymer is biodegradable, biocompatible and can be loaded with drugs. These capsules were shown to be efficient in vitro as contrast agents for both 19 F MRI and ultrasonography. In addition, for in vivo applications, a poly(ethyleneglycol) (PEG) coating promotes stability and prolonged circulation. Being stealth, nanocapsules can accumulate passively into implanted tumors by the Enhanced Permeation and Retention effect. Alternatively, they could be targeted to the neovasculature, via the overexpressed ?v?3 integrins, by decorating the nanocapsules with RGD moieties. Concentration and immobilization of the agents on a surface may improve the contrast enhancement for both imaging modalities. We will present nanocapsule formulation and characterization, and will show promising in vivo results obtained for both ultrasonography and 19 F MRI.

8955-41, Session 9

Dendronized magnetic nano-objects for MRI and hyperthermia *(Invited Paper)*

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Some of the significant and most promising applications for inorganic nanoparticles (NPs) lie in the fields of biology and biomedicine. Due to their magnetic properties tuned by their shape and/or composition, superparamagnetic iron oxide NPs (SPIO) with appropriate surface chemistry can be used in numerous in vivo applications such as MRI contrast enhancement, hyperthermia treatment, cell sorting, drug delivery...

In that context, we propose a concept combining a dendritic coating of magnetic oxide nanoparticles with phosphonate anchors. Indeed, phosphonates ensure a strong anchoring at the NPs surface while preserving their magnetic properties, and dendritic shells, in addition to their small and easily controllable size (as a function of their generation), are promising building blocks simultaneously solving the problems of biocompatibility, large in vivo stability and specificity. Dendronized iron oxide nanoparticles were demonstrated to induce any cytotoxicity. In vivo and in vitro MRI measurements showed that the contrast enhancement properties of the dendronized NPs were higher than those obtained with commercial polymer-coated NPs. Moreover, both types of dendronized NPs were eliminated by urinary and hepatobiliary pathways without unspecific uptake especially in the RES organs and in the lungs. The design of dendronized NPs was further improved to obtain theranostic nano-objects (which can both identify disease states and simultaneously deliver therapy) by adjusting the morphology and the composition of the inorganic core and by designing multifonctionalized dendrons.

8955-42, Session 9

Investigation of magnetic field enriched surface-enhanced resonance Raman scattering performance using Fe3O4@Ag nanoparticles for malaria diagnosis

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We have previously demonstrated the development of magnetic field-enriched surface enhanced resonance Raman spectroscopy (SERRS) for early malaria diagnosis. This magnetic field-enriched SERRS strategy enhanced the Raman spectra of ?-hematin, which is equivalent to hemozoin (a malaria biomarker generated by malaria parasites) in Raman features, by using the nanoparticles with iron oxide core and silver shell (Fe3O4@Ag) to enable SERRS. Although the plasmonic properties related to the different core and shell sizes of other types of nanoparticles have been examined in the literature, the modified SERRS performance of these nanoparticles under an external magnetic field has never been evaluated before. Moreover, the paramagnetic ?-hematin crystal under investigation in this strategy is much larger than the commonly used test molecules in surface-enhanced Raman spectroscopy such as

Rhodamine 6G that are non-magnetic, which further complicates the optimization of nanoparticles for SERRS.

In this work, we investigate the dependence of magnetic field enriched SERRS performance of ?-hematin on the core and shell sizes of Fe3O4@Ag nanoparticles. We note that Raman enhancement is improved in Fe3O4@Ag with a larger Fe3O4 core and a thicker Ag shell in the absence of a magnetic field. On the contrary, in the presence of an external magnetic field, Fe3O4@Ag with a smaller Fe3O4 core and a thinner Ag shell lead to the formation of more aggregates and hot spots that render a stronger Raman enhancement. These results are consistent with our simulations that will guide the optimization of magnetic SERRS for early malaria diagnosis.

8955-43, Session 9

Iron-oxide colloidal nanoclusters: from fundamental physical properties to diagnosis and therapy *(Invited Paper)*

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Research on magnetic nanocrystals attracts wide-spread interest due of their challenging fundamental properties, but it is also driven by problems of practical relevance and importance to the society, ranging from electronics (e.g. magnetic recording) to biomedicine. In that respect, iron-oxides are ideal functional materials as they adopt a variety of oxidation states and coordination environments which facilitate their use. In their nanoscale form, size and shape controlled iron-oxide nanoparticles exhibit different magnetic behavior from that in the bulk and emerge with great biomedical potential, especially with the aid of solution-phase colloidal synthesis. In this area, the magnetic field manipulation of the surface-modified iron-oxide nanoparticles impacts their utilization as carriers for drug delivery, in cell separation or even as localised heat sources and magnetic resonance imaging (MRI) contrast agents. We show that a promising way to engineer further their technological potential in diagnosis and therapy is by assembling primary nanocrystals into larger colloidal entities, possibly with increased structural complexity. In that context, elevated-temperature nanochemistry (e.g. polyol) approaches permitted us to develop size-tunable, low-cytotoxicity iron-oxide nanoclusters, entailing iso-oriented nanocrystals, with enhanced magnetization against that of individual particles. The clusters’ optimized magnetic anisotropy (including microscopic surface spin disorder as well as exchange interactions) and weak ferrimagnetism at room temperature, while they do not undermine colloidal stability, endow them a profound advantage in their efficacy as MRI contrast agents and hyperthermic mediators.

8955-44, Session 10

Direct observation of specific uptake of individual fluorescing gold nanoparticles *(Invited Paper)*

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In order to use nanoparticles as optical labels for biological applications on a cellular level both, the optical properties as well as the specific biological interaction needs to be known. Here, we report the application of gold nanoparticles (having diameters from few to several tens of nanometers) as labels for antibodies or aptamers binding to the Interleukin 6 receptor as present at the surface of mammalian cells. The conjugates are additionally covered with bovine serum albumin (BSA) or poly ethylene glycol (PEG) to avoid unspecific uptake, while specific binding is ensured by the specific recognition elements. We found that several parameters such as incubation time and temperature play an important role to the uptake rates.

In parallel we present a detailed study on the fluorescence mechanism of gold particles, to further use them as optical labels. We show that the fluorescence of small particles is governed by surface properties while particles of more than 10 nm in diameter show fluorescence due to inter band transitions. To finally image the binding events we compare optical scattering and PL images with scanning electron microscopy to directly count the number of gold nanoparticles on the cell surface. As a result, we could clearly distinguish between specific or unspecific interaction of the particles proven by the analytical investigation of the gold amount in or on the cells.

8955-45, Session 10

Specific markers, micro-environmental anomalies and tropism: opportunities for gold nanorods targeting of tumors in laser-induced hyperthermia

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Gold nanorods (GNRs) are optimal contrast agents for near-infrared (NIR) laser-induced photothermal ablation of cancer. Selective targeting of cancer cells can be pursued by attaching specific molecules on the particles surface or by the use of cellular vectors loaded with GNRs. We performed and tested various targeting approaches by means of GNRs functionalization with (i) antibodies against Cancer-Antigen-125 (CA-125), (ii) inhibitors of the carbonic anhydrase 9 (CA9) and (iii) by the use of macrophages as cellular vectors.

GNRs with a NIR absorption band at 810 nm were synthesized and PEGylated. For GNRs functionalization the targets of choice were CA-125, the most widely used biomarker for ovarian cancer, and CA9, overexpressed by hypoxic cells which are often located within the tumor mass. In the case of cellular vectors, to be used as Trojan horses naturally able to reach tumor areas, the surface of PEG-GNRs was modified to achieve unspecific interactions with macrophage membranes. In all cases the cellular uptake was evaluated by silver staining and spectrophotometric analysis. Cell viability was assessed by MTT test.

Then tests of laser-induced GNRs-mediated hyperthermia were performed in various cell cultures illuminating with an 810 nm diode laser (CW, 0,5-4 W/cm2 power density, 1-10 min exposure time) and cell death was evaluated. Each targeting strategy we tested may be used alone or in combination, to maximize the tumor loading and therefore the

efficiency of the laser treatment. Moreover, a multiple approach could help when the tumor variability interferes with the targeting directed to a single marker.

8955-46, Session 10

Magneto-plasmonic nanoclusters for capture and photoacoustic detection of cancer cells

Chun-Hsien Wu, Jason R. Cook, Stanislav Y. Emelianov, The Univ. of Texas at Austin (United States); Konstantin V. Sokolov, The Univ. of Texas at Austin (United States) and M.D. Anderson Cancer Ctr. (United States)

Nanoparticle technology provides advanced platforms for many biomedical applications such as tumor targeting, imaging, and delivery of drugs. Particularly, nanoparticle probes with multiple functionalities are highly desirable. However, integration of multiple components in a single nanostructure is a challenging task. Most of the existing approaches to synthesis of hybrid nanoparticles require cumbersome multi-step protocols and result in nanostructures with limited tunability of physical and optical properties. Here, we developed a new type of hybrid magneto-plasmonic nanoclusters with high absorbance in the near-infrared region and a strong magnetic moment. The nanoclusters consist of primary 6 nm iron oxide core-gold shell nanoparticles and are synthesized using oil-in-water microemulsion method. This method results in highly uniform spherical nanoparticles with sizes that can be varied from 90 to 180 nm. The nanoclusters combine the advantages of iron oxide and gold nanoparticles, enabling multiple useful properties such as a high magnetic moment and a high contrast in dark-field optical imaging, MRI and photoacoustics. The nanoclusters were functionalized with monoclonal antibodies for molecular specific detection of cancer cells using optical and photoacoustic imaging modalities. Furthermore, paramagnetic properties of the molecular targeted nanoclusters allowed efficient separation of cancer cells from whole blood using a magnetic field gradient. Thus, the hybrid magneto-plasmonic nanoclusters provide a promising platform for simultaneous capture and enumeration of circulating tumor cells (CTCs) in whole blood using magnetic cell sorting combined with photoacoustic detection in the near infrared region where the background signal from red blood cells is negligible.

8955-47, Session 10

Multimodal near-IR contrast agents for immune cell tracking

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Tracking of immune cells can provide better mechanistic understanding of various pathologies including cancer and new insights into therapeutic interventions. Moreover, using immune cells as transport vehicles for imaging and therapeutic agents can be beneficial in imaging and treatment of cancer in avascular regions. As biological tissues adsorb light in the visible spectral range, development of near-infrared contrast agents is essential for cell tracking. Gold nanorods are attractive candidates for in-vivo tracking of multiple cell populations for several reasons – high optical cross-sections, tunable absorbance, chemical inertness and biocompatibility. Furthermore, strong optical absorption of gold nanorods provides high imaging contrast in photoacoustic (PA) imaging. However, optical spectra of gold nanorods broaden upon cellular uptake due to plasmon resonance coupling; this renders simultaneous imaging of cell populations labeled with different nanorods difficult due to significant increase in spectral overlap. To address this problem, we have synthesized silica-coated gold nanorods with fluorescent dyes embedded in the silica matrix by either physical encapsulation or a covalent linking approach and demonstrated that plasmon resonance coupling can be circumvented by the silica coating. The fluorescent molecules provide possibility for fluorescent imaging using animal models with an implanted optical window and facilitate ex-vivo tissue evaluation. We demonstrated a good cellular uptake of silica coated gold nanorods by two macrophage cell lines with no cytotoxicity up to two days. PA imaging of tissue-mimicking phantoms with nanorod-loaded macrophages showed linear increase in the PA signal with number of cells and the sensitivity of just five cells per imaging kernel.

8955-48, Session 10

DNA as molecular local thermal probe for magnetic hyperthermia analysis

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Magnetic nanoparticles when coupled to an alternating magnetic field are capable of energy absorption and then release it in form of heat,[1] which can be used as a tool in many bioapplications.[2,3] The determination of temperature increments around excited nanoparticles represent however an experimental challenge and existing reports tend to show simple and limited assessments.[4,5] We demonstrate experimentally these temperature increments, when 12 nm magnetic nanoparticles are exposed to a radiofrequency radiation. Moreover, by functionalizing the surface of the nanoparticles with DNA molecules and further hybridizing with different length fluorophore-modified DNA an accurate temperature spatial mapping could be determined. Due to the design of these DNAs, different denaturalization temperatures (melting temperature, Tm) could be achieved. The quantification of the denaturalized fluorophore-modified DNA, and by interpolation onto a Boltzmann fitting model, it has been possible to calculate the local temperature increments at different distances, corresponding to the length of each modified DNA, from the surface of the nanoparticles. The local increments achieved were up to 15ºC, and the rigidity conferred by the double strand DNA allowed to evaluate the temperature at distances up to 5.6 nm from the nanoparticle surface.

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8955-49, Session 11

Internalization and functionalization of bio-compatible chitosan gold nanoparticles in NG108 neuronal cells

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With continued interest in gold nanoparticles (AuNPs) in biomedical applications, it is important to understand the uptake process and particle localization in cells. The added attribute of functional nanoparticles coatings, such as bio-compatible polymers, makes these particles attractive as carriers for drug delivery applications. In this study, we monitor the cellular uptake of bio-compatible chitosan-stabilized gold nanoparticles (Chit AuNPs) synthesized in the presence of tripolyphosphate (TPP) and a monovalent salt. We utilize both TPP and monovalent salt for minor control of shape and size dispersity. The resulting AuNPs are introduced to NG108 neuronal cells, that when activated at their plasmonic wavelength, have been shown to initiate the cell differentiation process of NG108 neuronal. Determination of cell growth and viabilty with the Chit AuNPs, a luminescence measurement using celltiter glo (CTG) is performed to measure ATP, which is only present in viable cells. Additionally, due to the particle having a small coating of chitosan on the surface, available functional groups are labeled and used for imaging with confocal and stimulated emission depletion (STED) microscopy. The basis for use of the STED microscopy is that it is possible to image utilizing the labeled the functional groups of the chitosan such that the plasmons of the gold particles are not cross emitting, removing any signal interference. Results of this work will be used for later studies in laser induced laser activation studies.

8955-50, Session 11

Gold nanoparticles based colorimetric nanodiagnostics for cancer and infectious diseases

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Traditional in vitro diagnostics requires specialized laboratories and costly instrumentation, both for the amplification of nucleic acid targets (usually achieved by PCR) and for the assay readout, often based on fluorescence. We are developing hybrid nanomaterials-based sensors for the rapid and low-cost diagnosis of various disease biomarkers, for applications in portable platforms for diagnostics at the point-of-care. To this aim, we exploited the size and distance-dependent optical properties of gold nanoparticles (AuNPs) to achieve colorimetric detection. Moreover, in order to avoid the complexity of thermal cycles associated to traditional PCR, the design of our systems includes signal amplification schemes, achieved by the use of enzymes (nucleases, ligase, helicase) or DNAzymes. Focused on instrument-free and sensitive detection, we carefully combined the intrinsic sensitivity by multivalency of functionalized AuNPs with isothermal and non-stringent enzyme-aided reaction conditions, controlled AuNPs aggregates, universal reporters and magnetic microparticles, the latter used both as a substrate and as a means for the colorimetric detection1. We obtained simple and robust assays for the sensitive (pM range or better) naked-eye detection of cancer or infectious diseases (HPV) biomarkers, requiring no instrumentation except for a simple heating plate. Finally, we demonstrated also non-medical applications of these bio-nanosensors, such as in the development of on-field rapid tests for the detection of toxic metals pollutants and other food and water contaminants.

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8955-51, Session 11

Spatiotemporal control of signaling pathways using magnetic nanoparticles *(Invited Paper)*

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Many biological systems require precise positional information to function correctly. This positional information is often encoded in protein concentration gradients and can contribute to many biological functions such as the regulation of the morphogenetic properties of cell organelles. For instance, one mode of organization recently proposed to contribute to the morphological properties of the mitotic spindle concerns the existence of concentration gradients of key regulatory proteins that feedback on microtubules and motors. Recent biochemical experiments and microscopic observations enabled the discovery of intracellular gradients of the active form of GTPase Ran, generated by a guanine exchange factor, RCC1 and involved in the spatiotemporal regulation of microtubule. Despite these progresses, the mechanisms controlling such morphogenetic gradients and the self-organizing properties of cytoskeletal elements are still unclear. To dissect these mechanisms one need to develop novel strategies based on the perturbations of protein reactivity. We are using magnetic nanoparticles to spatially localize regulatory proteins and interfere with intracellular morphogenetic signals. Superparamagnetic nanoparticles have been selected for their ability to be directed through eukaryotic cytoplasm when submitted to a magnetic field. Using Xenopus egg extracts we have developed an in vitro system capturing the spatial organization of cytoskeleton filaments within a geometry that mimic the cellular environment. We found that microtubule nucleation can be induced by magnetic accumulation of RanGTP bound to nanoparticles. The microtubule based structure assembles into an asymmetric array of polarized fibers that have their orientation dictated by the direction of magnetic forces. We found that spatial localization of RCC1 bioconjugated to nanoparticles induce microtubule assembly with a long-range distance effect. These results combined with a model based on reaction diffusion processes illustrate how signalling cascades could convey positional information over cellular scale. Our approach demonstrates how nanoparticles can be used to engineer signalling pathways and spatial self-organization.

8955-52, Session 11

Intracellular light-induced release of signaling molecules from gold-coated liposomes

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The combination of laser light and composite nanovesicles enables unique opportunities for precise delivery to, and on-demand release of molecular compounds within, single cells at high spatiotemporal resolution. Here, we demonstrate precise delivery and release of intracellular signaling molecules from gold-coated liposomes via manipulation with near infrared light. The plasmon resonant gold shell provides a light-sensitive trigger for on-demand content release from thermosensitive liposomes, as well as a highly polarizable nanostructure for strong interaction with an optical trap. Two schemes for intracellular delivery and release of liposome-encapsulated cell signaling molecules are presented here in the context of interrogating cell signaling pathways. The first uses a highly focused optical trapping beam to force gold-coated liposomes into a single cell and dump their contents intracellularly to elicit a calcium response. We demonstrate this optical delivery and release method using encapsulated inositol trisphosphate (IP3), a ubiquitous secondary messenger in cell signaling cascades, or adenophostin A (AdA), a potent IP3 analog with 10 to 100 times greater affinity to the IP3-receptor. The second delivery and release scheme preloading cells with gold-coated liposomes encapsulating IP3 or AdA, followed by illumination with a near infrared laser beam with a footprint colocalized to the liposomes-loaded cell. Exposure to this on-resonant wavelength of light induces rapid release of IP3 or AdA from the intracellular liposomes and subsequent activation of calcium signaling. These techniques are then applied to a 3D cell culture model of the tumor microenvironment to study communication involved in the development of cancer.

8955-53, Session 12

Insights into the cellular response following photothermal therapy *(Invited Paper)*

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Among many distinct nanomaterials, plasmonic metal nanoparticles are very prolific in literature, mainly due to their fascinating optical properties. Importantly, the NIR activity of plasmonic materials such gold nanoprisms (NPRs) makes them very useful materials for many relevant applications in vivo such as photothermal therapy and optoacoustic imaging, among others. In vitro studies are however a priority in order to assess their biosafety. Herein, we report a multiparametric investigation on the impact of surface modified NPRs on mice and human primary and transform cell lines. Upon exposure of NPRs to cells, and following uptake, most important parameters such as generation of reactive oxygen species (ROS), mitochondrial hyperpolarization, proliferation, cell morphology and apoptosis features were systematically assayed. Results clearly demonstrate a significant impact of the internalized NPRs in the cell morphology and mitochondria, which however do not translate into impartment of cell viability. Under NIR illumination, these nanoprobes can be triggered to cause apoptosis. Herein, molecular details following photothermal therapy are described.

8955-54, Session 12

Noninvasive high-speed imaging of fast physiological processes in awake and freely moving mice *(Invited Paper)*

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Non-invasive imaging of physiological processes like heart rate, breathing motion, blood perfusion and dynamic behavior of the gastro-intestinal tract are of great interest for preclinical research related to cardiovascular disease, hypertension or behavioral sciences.

So far imaging techniques available to study these processes with high spatial and temporal resolution require animals to be fixed and anesthetized. This has great limitations in terms of experiment time. More importantly anesthesia disturbs many physiological processes.

SWIR or NIR-II imaging (between 900 nm and 1700 nm) had been shown promising for non-invasive imaging. However, due to the suboptimal emission properties of the previously used materials (e.g. single walled carbon nanotubes (SWCNTs)) fast imaging rates of 25 to 50 frames per second could not be archived.

Here we use InAs/CdZnSe core-shell quantum dots, which emit in the SWIR region with a quantum yield of 30% after functionalization in physiologic solution. This emission intensity is two orders of magnitude higher than that of SWCNTs used previously for non-invasive SWIR imaging. Moreover, these quantum dots emit enough signal to study physiological processes in active mice opening a novel route to circumvent experimental artifacts generated by anesthesia.

For the first time we can facile image physiological parameters like heart rate, breathing rate, perfusion and energy metabolism in conscious and freely behaving mice.

8955-55, Session 12

Noninvasive detection of cervical cancer based on blood plasma surface-enhanced Raman spectroscopy

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Based on blood plasma surface-enhanced Raman spectroscopy (SERS) analysis, a simple and lable-free blood test for non-invasive cervical cancer detection was present in this paper. Silver nanoparticles as the SERS-active nanostructures were directly mixed with blood plasma to enhance the Raman scattering signals of various biomolecular constituents such as proteins, lipids, and nucleic acids. High quality SERS spectrum from blood plasma–Ag NP mixture can be obtained within 10 s using a Renishaw micro-Raman system. SERS measurements were performed on 60 cervical cancer patients and 50 healthy volunteers' blood plasma samples. Tentative assignments of the Raman bands in the measured SERS spectra suggest interesting cancer specific biomolecular differences as compared to that of healthy subjects. Principal components analysis (PCA) and linear discriminant analysis (LDA) were employed to analyze and classify the obtained blood plasma SERS spectra. The diagnostic algorithms based on PCA-LDA yielded a high diagnostic sensitivity of 96.7% and specificity of 92 % for separating cancerous samples from normal samples. This exploratory work demonstrated great potentials for developing SERS blood plasma analysis into a novel clinical tool for non-invasive detection and screening of cervical cancers.

8955-56, Session 12

Quantitative visualization and evaluation of size effect on cellular uptake of spherical gold nanoparticles (AuNPs) by using multiphoton laser scanning microscopy *(Invited Paper)*

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With ever-increasing applications of nanoscale materials in biomedical field, the impact of nanoparticle size on cellular uptake efficiency, dynamics and mechanism has attracted numerous interests but the knowledge in this aspect is still a matter of controversy. In this study a combined "multiphoton imaging-UV/Vis spectroscopic analysis" method was applied for the first time for quantitative visualization and evaluation on the cellular uptake process of different sized (15nm, 30nm, 50nm, 80nm) gold nanoparticles (AuNPs). This technique enables quantitative analysis of the size effect on cellular uptake behavior of AuNPs from a stack of 3D multiphoton laser scanning microscopy (MP-LSM) images meanwhile provides a capability for differentiating AuNPs present in external and internal subcellular components (cell membrane and cytoplasm) respectively, giving detailed information for better elucidating the cellular uptake mechanisms and dynamics. The data shows the internalization extent of AuNPs is highly dependent on particles sizes and incubation time. Due to sedimentation effect, 50nm and 80nm AuNPs are taken up by cells far more than 15nm and 30nm particles after exposure to cells for 24 h. However, the latter's uptake velocity is significant faster than the former before 10 h, indicating a disparity in uptake kinetics for different sized AuNPs. The finding from this study will improve our understanding in the cellular uptake mechanisms of different sized nanoparticles and have great implications in developing AuNPs-based drug carriers with various sizes for different purposes.

8955-57, Session 12

Engineering apoferritin nanocages for intracellular delivery of chemotherapeutics at cancer cells *(Invited Paper)*

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Nowadays, increasing attention is devoted to the use of nanoparticles, i.e., organic, inorganic or both, as drug delivery systems to improve the efficacy of chemotherapeutics and concomitantly reduce the well-known side effects associated with chemotherapies. However, while mechanistic insights into the improvement of cytotoxic activity of nanoformulated drugs are generally missing, they are still highly desired. In this study, an engineered apoferritin variant consisting of 24 heavy-chain subunits (HFn) was produced with the aim to achieve a cumulative delivery of antitumor drugs, which exert their cytotoxic action by integrating themselves in the DNA double strand, at the nucleus of human cancer cells. The rationale of our approach is based on exploiting the natural arsenal of defense of cancer cells to stimulate them to recruit large amounts of artificial apoferritins loaded with doxorubicin inside their nucleus in response to an oxidative stimulus, which results in a DNA damage leading to cell death. After demonstrating a remarkable selectivity of HFns for representative cancer cells compared with healthy fibroblasts, doxorubicin-loaded HFns (HFn-DOX) were used to treat the cancer cells. We show that HFn-DOX are internalized in cancer cells in a faster and more efficient way compared with free doxorubicin and we carefully investigate the potential interaction with different portals of entry, thus disclosing the pathway of internalization of the engineered nanocarrier. Our approach proved reliable and straightforward providing an antiproliferative effect with high reproducibility. Next, we explored the potential of combining the delivery of DOX with chemotherapeutics acting toward different targets, for instance, the anti-tubulinic agent docetaxel, assessing the synergistic effect of HFn-delivered combined chemotherapy. This strategy paves the way to the development of a new generation of localized combination therapy of cancer with a potential reduction of the well-known deleterious side-effects of standard chemotherapies.

8955-58, Session 12

Light-addressable amperometric electrodes for enzyme sensors based on direct quantum dot-electrode contacts *(Invited Paper)*

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Quantum dots allow the generation of charge carriers upon illumination. When these particles are attached to an electrode a photocurrent can be generated. This allows their use as a light-switchable layer on the surface. The QDs can not only exchange electrons with the electrode, but can also interact with donor or acceptor compounds in solution providing access to the construction of signal chains starting from an analyte molecule.

The magnitude and the direction of the photocurrent depend on several factors such as electrode polarization, solution pH and composition. These defined dependencies have been evaluated with respect to the combination of QD-electrodes with enzyme reactions for sensorial purpose.

CdSe/ZnS-QD-modified electrodes can be used to follow enzymatic reactions in solution based on the oxygen sensitivity. In order to develop a photoelectrochemical biosensor, e.g. glucose oxidase is immobilized on the CdSe/ZnS-electrode. One immobilization strategy applies the layer-by-layer-technique of GOD and a polyelectrolyte. Photocurrent measurements of such a sensor show a clear concentration dependent behaviour. The principle of combining QD electrodes with a layered architecture and light triggered read-out can also be transferred to other enzymes such sarcosine oxidase. The sensitivity of quantum dot electrodes can be influenced by additional nanoparticles, but also by multiple layers of the QDs.

In another direction of research it can be demonstrated that direct electron transfer from excited quantum dots can be achieved with the redox protein cytochrome c. This allows the detection of the protein, but also interaction partners such as enzymes or superoxide.

8955-71, Session 12

Poly(dopamine) capsosomes as advanced cell mimics: performing confined (cascade) enzymatic reactions in parallel *(Invited Paper)*

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The creation of simplified synthetic cells by assembling multicompartment systems, is envisioned to be an efficient tool with potential applications in the fields of drug delivery, (bio)sensing and diagnostics.[1] Considerable progress has been achieved in the assembly of functional, bio?inspired systems which tackle the challenge of mimicking cells by encapsulating multiple compartments and performing enzymatic reactions. Amongst them, we recently pioneered capsosomes, which is the combination of two intrinsically different components: multiple liposomes within a polymeric carrier capsule. [2] Although capsosomes have demonstrated to be one of the more advanced compartmentalized systems reported to date, with control over the amount[3] and position[4] of the liposomal compartments within the carrier capsule, and with the ability to perform confined triggered[3, 5] and continuous[4] enzymatic reactions, challenges regarding both their assembly and their functionality still need to be overcome. The fabrication of the carrier capsule relies on the deposition of interacting polymers via the Layer?by?Layer technique, which involves multiple polymers and sequential deposition steps. To reduce the high amount of labor and cost, herein, we report on the fabrication of a new generation of capsosomes consisting in liposomes embedded within a poly(dopamine) (PDA) carrier shell created in a solution?based single?step. The polymerization of dopamine into PDA is a fast and spontaneous process which has been recently reported for the formation of capsules with potential as drug delivery vehicles or for encapsulated catalysis.[6] Since biological cells are able to perform multiple, (cascade) enzymatic reactions simultaneously, we verify the potential of PDA?capsosomes as artificial cell mimics by performing a two?enzyme coupled reaction in parallel with a single?enzyme reaction by encapsulating three different enzymes into separated liposomal compartments and converting the substrate uric acid, which is a clinically valuable diagnostic indicator, into a fluorescent product. Taken together, our results demonstrate the potential of these newly designed capsosomes toward biosensing applications while, at the same time, we bring them a step closer to advanced biomimicry tools by performing simultaneously multiple functions within their confined compartments.

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8956-38, Session PSun

MGdLuF3:Nd3+ (M=K,Na) nanoparticles for trimodal medical imaging

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Near infrared light (NIR) based detection and therapy is a developing field in the biomedical industry. In this project, we are proposing the development of NIR based nanoparticles (NPs) with multimodal features for medical imaging. The multimodal imaging comprises optical imaging, magnetic imaging, and X-ray imaging by utilizing the superparamagnetic features of Gd and the high X-ray excitation cross section of Lu. Halides such as MGdLuF3 (M=K,Na) were doped with NIR active rare earth ions, Nd3+ where synthesis conditions have been optimized for obtaining the brightest phosphor with a size of < 50 nm. Characterizations of the NPs were done to explore the excitation and emission properties, crystal structure, TEM images, and magnetization properties. The nanophosphors were then coated with poly(maleic anhydride- alt-1-octadicene) (PMAO) to implement in confocal cellular imaging and cellular toxicity to determine the viability and cytotoxicity at different concentrations. Future work for the application of these nanophosphors in medical imaging will be confirmed by magnetic resonance (MRI) as well as positron emission tomography (PET).

8956-39, Session PSun

Partitioned carbon nanotubes as perspective nanomaterial for energy conversion

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Among the large variety of the modern materials of nano- and bioelectronics the carbon nanostructures of the complex form are one of the most perspective materials. In particular, the field-emission cathodes on a basis of these structures can be used in the medical radiography. Also the carbon nanostructure of the complex form can be applied at the design of the medical electronic equipment. For example, they can be used in a work of the field emission display. Results of the theoretical investigations of the emission properties for the partitioned (bamboo-like) carbon nanotubes are presented in this paper.

Theoretical investigation of the field emission properties for the partitioned nanotubes is carried out by the quantum-chemical tight-binding method. Partitioned nanotubes were modeled by joining of the fullerene fragment by chemical bonds to the inner surface of nanotube. The object of investigation in this work is partitioned nanotube (15,15), which is a stable bamboo-like nanotube of the smallest diameter.

It is established that the infinite partitioned nanotubes with diameter of 2.02 nm and distance between the bridges of 2.56 nm and 2.81 nm exceeds the emittance of the infinite hollow nanotubes. Consequently, infinite partitioned nanotubes with increasing distance between the bridges are superior on the emission properties of the hollow nanotubes

Thus one can conclude that the carbon partitioned nanotubes can be applied as nanoemitters with high field emission properties. Films with similar structures can be used as cathodes ensuring a stable current under small voltage in nano- and mictroelectronic devices.

8956-40, Session PSun

Unit coefficient of thermal conductivity of carbon nanotubes with positions of their use as a material for nano-emitters

Olga E. Glukhova, Anna S. Kolesnikova, Michael M. Slepchenkov, NG Chernyshevsky Saratov State Univ. (Russian Federation); Vladislav V Shunaev, NG Chernyshevsky Saratov State Univ (Russian Federation); Georgy V. Savostyanov, NG Chernyshevsky Saratov State Univ. (Russian Federation)

Currently, active research is conducted to develop field-electron emitters based on carbon nanotubes (CNT). It is known that the surface of the carbon nanotube is heated in the field emission process. This is the reason for the rapid destruction of the emitter based on CNT. Therefore urgent task is investigation of conductivity of nanotubes to reduce their heating and prevent their destruction.

Experimental study of the thermal conductivity of isolated CNT is time consuming and expensive process. Computational models have been developed for these reasons. Computational models help to better understand process of heat transfer in carbon nanotubes. Molecular dynamic (MD) method is one of the most effective methods for studying the processes occurring in carbon nanostructures. MD method can be used to study the thermal properties of CNTs different configuration (chirality, length, number of layers, the presence of any defects). Program packet was developed in Python. Calculation of the coefficient of thermal conductivity in the developed program was carried out by the Green-Kubo formula.

It is found that the thermal conductivity of the tube increases with the tube length. It is found that the thermal conductivity of the nanotube armchair (10, 10) with the length 28Å decreases with increasing number of defects Stone-Wales to 5. The optimal structure of CNTs, for which the maximum value of the coefficient of thermal conductivity is achieved, is the defect-free nanotube length of at least 45 Å. Consequently, ideal nanotubes without defects in their structure can be used as nanoemitters.

8956-41, Session PSun

Theoretical investigation of bilayer fullerene C60@C540 in term of its biomedical application

Olga E. Glukhova, Anna S. Kolesnikova, Michael M. Slepchenkov, Vladislav V. Shunaev, NG Chernyshevsky Saratov State Univ. (Russian Federation)

Due to a wide range of physical and chemical properties and multiformity of their structural types double layer fullerenes with the non-center effect may be applied in different fields of medicine, in particular for

drug delivery system. The aim of this study is to investigate the atomic structure and the calculation of a multi-well potential of interaction between nanoparticle layers in two-layer fullerene ?60@?540.

We have simulated the movement of the fullerene C60 inside the icosahedral fullerene C540. The numerical experiment was carried out by the method of molecular dynamics. The surface of the interaction energy for fullerenes was calculated. It was shown that the centers of fullerenes do not coincided.

Since fullerene C540 has an icosahedral symmetry, there are three types of potential wells inside its shell (and the fullerene C60 is able to take each of them): E1 = – 1,19 eV, E2 = – 1.16 eV, E3 = – 1,15 eV.

The considered model of bilayer fullerene ?60@?540 allows us to suggest that the application of external conditions (temperature) will make fullerene ?60 to move in a certain way: between the potential wells. It is obvious that with the increase of temperature the frequency hopping will increase too. Probably, the phenomenon of the ?60 jumping in a cage of other fullerene can be used in the current technologies, for example for determination of the local temperature by means of the increase of the jumps velocity.

8956-42, Session PSun

Microfabrication of curcumin-loaded microparticles using coaxial electrohydrodynamic atomization

Shuai Yuan, Ting Si, Univ. of Science and Technology of China (China); Zhongfa Liu, Ronald X. Xu, The Ohio State Univ. (United States)

Encapsulation of drugs in biodegradable polymers becomes attractive due to sustained and controlled drug release for treatment of diseases. So far many microencapsulation processes have been explored for fabricating drug-loaded microbubbles. However, commonly used processes such as double emulsion are limited by their low encapsulation rate, large size distribution, and potential damage of antibody/gene bioactivity. A coaxial electrohydrodynamic atomization (also known as 'coaxial electrospray') process has the potential to overcome the above limitations. In this work, the encapsulation of curcumin in PLGA microparticles is performed by the process. By introducing an elevated electric field between a coaxial capillary needle and ground, the contributing factors to flow instability, such as electrical intensity and liquid flow rates are tested experimentally. A drug delivery mode platform is also developed for the release of curcumin-loaded PLGA microparticles. To optimize the process, the effects of different process parameters and material properties on morphology and size distribution of resulting microparticles are studied systemically. The drug-loading efficiency is also checked and further compared with previous observations. The present system is advantageous for the enhancement in particle size distribution and drug-loading efficiency, and also for sustained and controlled drug release of curcumin-loaded PLGA microparticles.

8956-1, Session 1

Application strategies of photo-immunotherapy (PIT) for treating solid cancers *(Invited Paper)*

Hisataka Kobayashi, National Institutes of Health (United States)

Photo-immunotherapy (PIT) is a newly developed, molecularly-targeted cancer photo-therapy based on conjugating a near infrared silica-phthalocyanine dye, IR700, to a monoclonal antibody (MAb) thereby targeting cancer-specific cell-surface molecules. When exposed to NIR light, the conjugate induces a highly-selective necrotic cell death only in receptor-positive MAb-IR700-bound cancer cells. Necrosis occurs as early as 1 minute after exposure to NIR light and results in irreversible morphologic changes including cellular swelling, bleb formation, and rupture of vesicles due to membrane damage. Meanwhile, immediately adjacent receptor-negative cells are unharmed. Due to the concentration gradient of MAb-IR700 leaking from vessels, PIT first causes necrosis in perivascular cancer cells resulting in dramatically enhanced vascular permeability with enhanced nano-particle delivery to cancer tissue, an effect termed "super-enhanced permeability and retention (SUPR)". The combination of PIT and SUPR effects can effectively treat a variety of solid cancers including inhomogeneous cancers and cancer stem-like cells by employing different targeting molecules (including but not limited to MAbs) and nano-sized anti-cancer drugs. In this presentation, preclinical examples of successful PIT, employing a variety of single and multi-target-PIT, combined with nano-sized cancer reagents will be discussed. The combination of PIT and nano-sized systemic therapies is especially well adapted for real world heterogeneous tumors containing both receptor positive and receptor negative cells. The implications for clinical translation will be discussed.

8956-2, Session 1

Upconversion luminescence targeted imaging of tumor xenografts in vivo

Majid Badieirostami, Conroy Sun, Lei Xing, Stanford Univ. (United States)

Near infrared (NIR) optical imaging has demonstrated significant potential as an effective modality for cancer targeted molecular imaging. Among various NIR probes currently under investigation, upconversion nanophosphors (UCNPs) possess great promise due to their anti-Stokes emission and sequential photon absorption which result in superior detection sensitivity and simple imaging setup, respectively. Here we investigate the utility of this imaging modality to detect tumor cells expressing the epidermal growth factor receptor (EGFR) using affibody functionalized nanophosphors and a custom built imaging system. Initially, photophysical properties of UCNPs, their linear luminescence responses versus their concentrations, and their depth resolving capability in a tissue-simulating phantom are examined. Finally, we demonstrate the use of bioconjugated UCNPs for targeting EGFR-expressing tumors both in vitro and in vivo. Our data suggests that NIR imaging with UCNPs can be used as a specific modality for noninvasive imaging of tumors.

8956-3, Session 1

Monitoring intraperitoneal metastases using microendoscopy

Bryan Q. Spring, Adnan O. Abu-Yousif, Akilan Palanisami, Xiang Zheng, Imran Rizvi, Zhiming Mai, Sriram Anbil, R. B. Sears, Lawrence B. Mensah, Ruth Goldschmidt, Sultan S. Erdem, Massachusetts General Hospital (United States) and Harvard Medical School (United States); Esther Oliva, Massachusetts General Hospital (United States); Tayyaba Hasan, Massachusetts General Hospital (United States) and Harvard Medical School (United States)

We are developing fluorescence microendoscopy and image analyses to guide and monitor treatment of multifocal tumor nodules. Traditional imaging modalities fail to detect residual tumor deposits that frequently cause disease recurrence. To help address this problem, we present an approach that utilizes fluorescence microendoscopy.

8956-4, Session 1

Real-time visualization of pancreatic leak using a chymotrypsin-activated fluorescent probe during pancreatic surgery

Takeaki Ishizawa M.D., Yasuteru Urano, Mako Kamiya, Masayo Sakabe, Suguru Yamashita M.D., Nobuhiro Harada M.D., Atsushi Shimizu M.D., Junichi Kaneko M.D., Taku Aoki M.D., Yoshihiro Sakamoto M.D., Yasuhiko Sugawara M.D., Kiyoshi Hasegawa M.D., Norihiro Kokudo M.D., The Univ. of Tokyo (Japan)

Background: Inability to visualize the leakage of pancreatic juice during surgery makes it difficult to prevent postoperative pancreatic fistula, which remains a life-threatening complication after digestive surgery.

Methods: A chymotrypsin probe (glutaryl-phenylalanine hydroxymethyl rhodamine green with trypsin) was designed and synthesized. Fluorescence images were obtained by the multispectral imaging system with the blue-filter setting (excitation, 445 to 490 nm; emission, 515 nm long-pass). 1) The chymotrypsin probe was sprayed on to filter papers that had been placed on the resected pancreatic stump in patients undergoing pancreatic resection. 2) In a swine model, the chymotrypsin probe was sprayed directly on the remnant pancreatic stump.

Results: 1) Fluorescence imaging using the chymotrypsin probe enabled rapid visualization of pancreatic leak on filter papers in 25 out of the 32 patients. The fluorescence intensity of pancreatic juice on filter papers correlated positively with its amylase levels ($r=0.684$, $P<0.001$). Postoperatively, symptomatic pancreatic fistula never developed in the remaining 7 patients, in whom pancreatic leak was unidentifiable by the chymotrypsin probe. 2) Following administration of the chymotrypsin probe on the pancreatic stump, pancreatic leak was visualized not only by the multispectral imaging system but by naked-eye examination through light-blocking glasses, which enabled surgeons to suture the site of leakage.

Conclusions: The chymotrypsin probe can visualize pancreatic leak during surgery. If this probe can be used directly on the pancreatic stump in the patient's abdominal cavity in the future, it may enable surgeons to close the pancreatic leak intraoperatively, improving the safety of pancreatic resection.

8956-5, Session 1

Gx1-conjugated endostar nanoparticle: a new drug delivery system for anti-colorectal cancer in vivo

Qian Zhang, Xidian Univ. (China) and Life Sciences Research Ctr., School of Life Sciences and Technology (China); Yang Du, Institute of Automation (China); Yaqian Li, Harbin Univ. of Science and Technology (China); Xiaolong Liang, Peking Univ. (China); Xin Yang, Jie Tian, Institute of Automation (China)

(1). Colo-205-Luc2-2C1 cells, which express luciferase gene and can be detected through BLI in vivo, was used for making colorectal xenografts in nude mice in our study system. (2). The peptide GX1 was conjugated to the NIR dye CW800 nanocapsules to make the molecular targeting probe. The BLI signal was first captured to detect the tumor location. Then the in vivo dynamic bio-distribution of GX1-conjugated CW800 and the unconjugated CW800 nanocapsules (as control) were evaluated through FMI. (3). For the drug treatment evaluation, endo alone and GX1-endo groups were given the corresponding drugs through intravenous tail injection. Endostar was coated with PLA(Poly lactic acid), and last amino acid of GX1 was conjugated to the Endostar-loaded PLA nanoparticles. The drug dosage was 10 mg/kg/day and was administrated for continuous 10 days. The control group was given an equal amount of 0.9% saline. In vivo BLI was carried out for every 3 days.

We successfully developed a tumor specific NIR fluorescent probe, GX1-conjugated CW800 nanocapsules, which can be used for tumor angiogenesis imaging and holds a potential for the future clinical translation. The new GX1-endo drug delivery system had a better tumor targeting and anti-tumor growth efficacy than endo alone.

8956-6, Session 2

Dual-tracer receptor concentration imaging using tracers with different tissue delivery kinetics

Kenneth M. Tichauer, Illinois Institute of Technology (United States); Mamadou Diop, Univ. of Western Ontario (Canada) and Medical Biophysics (Canada); Jonathan T. Elliott, Kimberley S. Samkoe, Dartmouth College (United States) and Thayer School of Engineering (United States); Tayyaba Hasan, Wellman Ctr. for Photomedicine, Harvard Medical School (United States); Keith St. Lawrence, Univ. of Western Ontario (Canada) and Medical Biophysics (Canada); Brian W. Pogue, Dartmouth College (United States) and Thayer School of Engineering (United States)

Simultaneous dynamic fluorescent imaging of a suitable untargeted tracer in conjunction with any molecular targeted fluorescent agent has been shown to be a powerful approach for quantifying cancer-specific cell surface receptors in vivo in the presence of non-specific uptake and tracer delivery variability. The identification of a "suitable" untargeted tracer (i.e., one having equivalent plasma and tissue delivery pharmacokinetics to the targeted tracer) for every targeted tracer, however, may not always be feasible or could require extensive testing. This work presents a "deconvolution" approach capable of correcting for plasma pharmacokinetic differences between tracers by functionalizing dynamic differences in targeted and untargeted tracer uptake in a reference tissue (one devoid of targeted molecular species) and correcting uptake in all other tissues accordingly. This deconvolution correction approach is explored in theoretical models and evaluated in an in vivo mouse xenograft model of human glioma. In the animal experiments, epidermal growth factor receptor (EGFR: a receptor known to be overexpressed in the investigated glioma cell line) was targeted using a fluorescent tracer with very different plasma pharmacokinetics than a second untargeted fluorescent tracer. Without correcting for these differences, the dual-tracer approach yielded substantially higher estimations of EGFR concentration in all tissues than expected; however, deconvolution correction was able to produce estimates that matched ex vivo validation.

8956-7, Session 2

Ultrasmall lanthanide-doped nanoparticles as multimodal platforms

Brian G Yust, Francisco J. Pedraza, Dhiraj K. Sardar, Univ. of Texas at San Antonio (United States)

Recently, there has been a great amount of interest in nanoparticles which are able to provide a platform with high contrast for multiple imaging modalities in order to advance the tools available to biomedical researchers and physicians. However, many nanoparticles do not have ideal properties to provide high contrast in different imaging modes. In order to address this, ultrasmall lanthanide doped oxide and fluoride nanoparticles with strong NIR to NIR upconversion fluorescence and a strong magnetic response for magnetic resonance imaging (MRI) have been developed. Specifically, these nanoparticles incorporate gadolinium, dysprosium, or a combination of both into the nano-crystalline host to achieve the magnetic properties. Thulium, ytterbium , and neodymium codopants provide the strong NIR absorption and emission lines that allow for deeper tissue imaging since near infrared light is not strongly

absorbed or scattered by most tissues within this region. This also leads to better image quality and lower necessary excitation intensities. As a part of the one pot synthesis, these nanoparticles are coated with peg, pmao, or d-glucuronic acid to make them water soluble, biocompatible, and bioconjugable due to the available carboxyl or amine groups. Here, the synthesis, morphological characterization, magnetic response, NIR emission, and the quantum yield will be discussed. Cytotoxicity tested through cell viability at varying concentrations of nanoparticles in growth media will also be discussed.

8956-8, Session 2

Bodipy and curcumin modified molecular contrast agent for photoacoustic imaging

Samir Laoui, Stephanie Bellinger Buckley, Olivier Dantiste, Univ. of Massachusetts Boston (United States); Jen-Chieh Tseng, Dana-Farber Cancer Institute (United States) and Lurie Family Imaging Ctr. (United States); Jonathan Rochford, Chandra S. Yelleswarapu, Univ. of Massachusetts Boston (United States)

Cancer is the most prevalent disease throughout the world. For successful diagnosis and treatment, a complete understanding of cancer cell growth is necessary. Recently photoacoustic imaging has been developed to aid in the understanding of cancer cell growth by providing tumor location and metabolic activity. Contrast agents used for photoacoustic imaging can be categorized into two types: natural present (endogenous) and synthetic administered (exogenous) contrast agents. Endogenous pigments, such as hemoglobin and melanin, show a strong absorption of visible light, by default are non-toxic and allow imaging without altering true physiological conditions. In many scenarios however, such as the detection of early stage tumors, an endogenous contrast agent alone is insufficient to provide enough information. In these cases, optimized exogenous contrast agents are frequently needed to provide better signal/contrast for photoacoustic imaging. We are developing molecular photoacoustic contrast agents (MPACs) through chemical modification of efficient and established fluorescent probes. Using a bottom up approach non-emissive functionalities are being conjugated to the well-known BODIPY and curcumin fluorescent probes so that the absorbed energy is directed towards a nonradiative excited-state decay pathway. Optical and photoacoustic characterization of our MPACs demonstrates a stronger photoacoustic signal compared to the corresponding fluorescent probes. The increased photoacoustic response of MPACs will enable tumor imaging at greater depths while avoiding hard matter nanoparticle contrast agents thus reducing potential toxicity effects which is a current concern of the research community.

8956-9, Session 2

Photothermal optical coherence tomography and therapy in targeted mouse brain tumors using gold nanostars

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Gold nanostars (AuNS) have strong absorption and scattering properties in the near-infrared light due to their unique plasmon response. These properties provide potential of designing novel optically active reagents for simultaneous molecular imaging and therapy. Its biocompatibility and ease of conjugation to proteins make AuNS more attractive as contrast agents.

Detection and resection of brain tumors is one of the main challenges in cancer therapy. The brain tumors do not have a clear boundary separating them from the adjacent normal brain tissues, and it is difficult to remove only desired region of brain. Therefore, imaging modalities that provide a clear visualization of cancerous tissues with sufficient imaging depth would impact the current brain cancer diagnosis and therapy.

Here, we propose photothermal optical coherence tomography (PT-OCT) and therapy based on AuNS for structural and molecular-targeted imaging of biological tissues. We performed PT-OCT imaging using phase-resolved optical frequency domain imaging system with AuNS, which has a peak absorption cross-section at 800nm. The pump beam modulation was provided by an 808nm laser diode with a power of 10mW and the frequencies up to 1000Hz. The PT induced phase modulation was measured with high signal-to-noise ratio (50dB at 100Hz) and structural images of mouse brain could also be obtained with high spatial resolution (~21um) and axial resolution (~6um).

In this talk, we will demonstrate photothermal imaging and therapy of AuNS-targeted mouse brain tumors. The imaging performance of PT-OCT system will be described, and the capability of PT therapy based on AuNS will also be evaluated.

8956-10, Session 2

One-step production of multilayered microparticles by tri-axial electro-flow focusing

Ting Si, Hanxin Feng, Yang Li, Xisheng Luo, Univ. of Science and Technology of China (China); Ronald X. Xu, Ohio State Univ. (United States)

Microencapsulation of multiple drugs and imaging agents in biodegradable microparticles has great applications in multimodal imaging and image-guided therapy. The most commonly used methods for microencapsulation is emulsion. Other techniques based on capillary flows have also been explored, such as microfluidic channel, coaxial electrospray, and coaxial flow focusing. However, most of the existing microencapsulation processes involve only two types of materials and produce simple core-shell structure. In this work, we present a tri-axial electro-flow focusing (TEFF) technique for one-step encapsulation of drugs and imaging agents in biodegradable multi-layered microparticles. The TEFF process can be characterized as a tri-axial cone-jet configuration in the core of a high-speed coflowing gas stream under an axial electric field. The tri-axial liquid jet eventually breaks up into multi-layered microcapsules. In experiments, the concentric stainless steel capillary needles were fabricated by laser welding technique and the concentricity of the three needles could be easily achieved. The TEFF device was developed and well validated using a set of experimental setup that was used in our previous studies. Stable cone-jet structures with three different interfaces were observed. The effect of main process parameters including the flow rates of three different liquids, the pressure difference, the electric potential and the geometric parameters on the multi-fluidic compound cone-jet configurations was studied. Different flow modes and corresponding regimes were also identified. The TEFF process yielded multilayered microparticles with uniform particle size distribution, large productivity, and high drug-loading efficiency.

8956-11, Session 3

Mediating the potent ROS toxicity of acrolein in neurons involved in secondary spinal cord injury with silica nanoparticles and a natural product approach *(Invited Paper)*

Desiree White-Schenk, Riyi Shi, James F. Leary, Purdue Univ. (United States)

Acrolein, a very reactive aldehyde, is a culprit in the biochemical cascade after primary, mechanical spinal cord injury (SCI), which leads to the destruction of tissue initially unharmed, referred to as "secondary injury". Additionally, in models of multiple sclerosis (MS) and some clinical research, acrolein levels are significantly increased. Due to its ability to make more copies of itself in the presence of tissue via lipid peroxidation, researchers believe that acrolein plays a role in the increased destruction of the central nervous system in both SCI and MS. Hydralazine, an FDA-approved hypotensive drug, has been shown to scavenge acrolein, but its side effects and short half life at the appropriate dose for acrolein scavenging must be improved for beneficial clinical translation. Therefore, a nanomedical approach has been designed using silica nanoparticles as a porous delivery vehicle hydralazine. The silica particles are formed in a one-step method that incorporates poly(ethylene) glycol (PEG), a stealth molecule, directly onto the nanoparticles. As an additional avenue for study, a natural product in green tea, epigallocatechin gallate (EGCG), has been explored for its ability to react with acrolein, disabling its reactive capabilities. Upon demonstration of attenuating acrolein, EGCG's delivery may also be improved using the nanomedical approach. The current work exposes the potential of using Cy5.5 labeled silica nanoparticles as a delivery vehicle and EGCG's antioxidant capabilities in B35 neuroblastoma cells exposed to acrolein. We also measure nanotoxicity to individual human neurons using high-throughput image scanning cytometry and confocal microscopy.

8956-12, Session 3

Nanoparticle-enhanced x-ray therapy for cancer

Renat R Letfullin, Rose-Hulman Institute of Technology (United States) and Radiological Technologies Univ. (United States); Colin E. W. Rice, Univ. of Minnesota (United States) and School of Physics & Astronomy (United States); Thomas F. George, Univ. of Missouri–St. Louis (United States); Marziya Yashkarova, Semey State Univ. named after Shakarim (Kazakhstan); Kunnaz Murzagulova, ROMAT (Kazakhstan)

The photothermal therapies of nanophotohyperthermia and nanophotothermolysis utilize the light absorptive properties of nanoparticles to create heat and free radicals in a small localized region. Conjugating nanoparticles with various biomolecules allows for targeted delivery to specific tissues or even specific cells, cancerous cells being of particular interest. Previous studies have investigated nanoparticles at visible and infrared wavelengths where surface plasmon resonance leads to unique absorption characteristics. However, issues such as poor penetration depth of the visible light through biological tissues limits the effectiveness of delivery by noninvasive means. In other news, various nanoparticles have been investigated as contrast agents for traditional X-ray procedures, utilizing the strong absorption characteristics of the nanoparticles to enhance contrast of the detected X-ray image. Using X-rays to power photothermal therapies has three main advantages over visible-spectra wavelengths: the high penetration depth of X-rays through biological media makes non-invasive treatments very feasible; the high energy of individual photons means nanoparticles can be heated to desired temperatures with lower beam intensities, or activated to produce the free radicals; and X-ray sources are already common throughout the medical industry, making future implementation on existing equipment possible. This paper uses Lorenz-Mie theory to investigate the light absorption properties of various size gold nanoparticles over photon energies in the 1-100 keV range. These absorption values are then plugged into a thermal model to determine the temperatures reached by the nanoparticles for X-ray exposures of differing time and intensity. The results of these simulations are then discussed in relation to the effective implementation of nanophotohyperthermia and nanophotothermolysis treatments.

8956-13, Session 3

Multiplexed detection of cell-surface cancer biomarkers with targeted SERS-coded nanoparticles

Yu Wang, Madhura Som, Altaz Khan, Ye Chen, Danni Wang, Jonathan T. Liu, Stony Brook Univ. (United States)

Our lab is developing a miniature endoscopic imaging device for in vivo detection of cell-surface biomarkers of cancer progression. In particular, this work employs targeted surface-enhanced Raman scattering (SERS) nanoparticles (NPs) to enable the sensitive and multiplexed detection of a large number of cell-surface biomarkers. The SERS NPs were functionalized with different targeting antibodies, and their biomarker detection capability was investigated via in vitro and ex vivo experiments with cells and tissues. Here, we design SERS NPs to specifically target the cancer biomarkers EGFR and HER2 upon topical application on cells and tissues. In preparation for future in vivo studies, we have developed a fiber-optic-based spectral detection probe, with 785-nm laser illumination, for rapid detection of SERS NPs with sub-millimeter spatial resolution. In vitro flow cytometry with fluorescent SERS NPs reveals a high ratio of specific versus nonspecific binding for the tumor cell lines A431 (EGFR-positive) and SkBr3 (HER2-positive). In vitro experiments with cell monolayers also demonstrate significant contrast between positive and negative NPs after multiple rinses to remove unbound probes. Finally, ex vivo experiments with xenograft tumor explants show the ability of multiplexed SERS NPs to detect cell-surface biomarkers when topically applied on tumor tissues. These studies indicate that antibody-functionalized SERS NPs may potentially be used as a sensitive and reliable contrast agents for the detection of a multiplexed panel of disease biomarkers.

8956-14, Session 3

Development of highly-sensitive fluorescent thermometer based on thermo-responsive polymers and nanoparticles for intracellular thermal imaging

Mingyuan Wei, Bingbing Cheng, The Univ. of Texas at Arlington (United States); Venugopal Bandiy, Univ. of North Texas (United States); Yuan Liu, The Univ. of Texas at Arlington (United States); Francis D'Souza, Univ. of North Texas (United States); Kytai T. Nguyen, Baohong Yuan, The Univ. of Texas at Arlington (United States)

Due to non-invasive measurement and the capability of large scale imaging, optical thermometers have been attracted great attention, such as infrared (IR) thermography, thermo-reflectance, optical interferometry, Raman thermometer, and fluorescence thermometer. Among them, fluorescent thermometer is of high resolution (~0.1 μm), high sensitivity, short acquisition time, diversity of detection method (intensity or lifetime), and compatibility in biological samples (e.g. cell or tissue). Thermo-responsive polymers have been demonstrated as an excellent substrate for carrying fluorophores in the development of "Switch-like" fluorescent thermometers (1). The intensity ratios between the ON and

OFF state were found ~10 but less than 20. Herein we report a new fluorescent thermometer based on a polarity-sensitive fluorophore (an Aza-BODIPY dye derivative) and thermo-responsive polymer poly (N-isopropylacrylamide) (PNIPAM) or its nanoparticles (NPs). When the fluorophore was covalently attached on the linear polymer, the intensity ratios were improved by more than one order of magnitude, where the intensity ratios are from ~300 to ~700. When the fluorophore was attached to the surface of NPs, the intensity ratio was found to be ~45. Furthermore, a large temperature range of ~20 °C was achieved if co-polymerized with hydrophilic monomers. Non-surprisingly, the temperature resolution, i.e. the slope of intensity-to-temperature, would be significantly improved. The findings in the present study pave the way for future intracellular thermal imaging work.

Reference.

1. X.-d. Wang, O. S. Wolfbeis and R. J. Meier, “Luminescent probes and sensors for temperature,” Chemical Society Reviews (2013)

8956-15, Session 4

Magneto-plasmonic nanoclusters with high-NIR absorbance for tracking, detection, and capture of rare cells

Konstantin V. Sokolov, Univ. of Texas M.D. Anderson Cancer Ctr. (United States); Chun-Hsien Wu, Jason Cook, Stanislav Emelianov, The Univ. of Texas at Austin (United States)

Detection of disseminated tumor cells or tumor biomarkers in human fluids such as blood, urine, and saliva can provide an accessible tool for cancer detection and monitoring. In particular, accurate quantitation of cancer cells in the bloodstream can help to determine prognosis and monitor the effectiveness of cancer therapy. However, the challenge of detecting circulating tumor cells (CTCs) is their rare occurrence, estimated as one to few CTCs among millions of leukocytes and billions of erythrocytes.

Here we address this challenging problem by combining technological advances in immunotargeted hybrid magneto-plasmonic nanoclusters and photoacoustic (PA) imaging. We developed a new type of nanoparticles which consist of primary 6 nm iron oxide core-gold shell nanoparticles that form highly uniform spherical assemblies with sizes that can be varied from ca. 70 to 180 nm. The magneto-plasmonic nanoclusters exhibit strong red-NIR absorbance and superparamagnetic properties with a high magnetic moment in an external magnetic field. We conjugated the nanoclusters with monoclonal antibodies specific for tumor biomarkers of breast and skin cancers and demonstrated molecular specific optical and PA imaging with high sensitivity. Furthermore, we showed that molecular targeted nanoclusters can be used for simultaneous magnetic capture and PA detection of cancer cells in whole blood with greater than 90% capturing efficiency with no laborious processing steps that are commonly used in other cancer cell capture and enumeration assays. In this talk we will explore the opportunities afforded by the hybrid magneto-plasmonic nanoparticles and PA imaging for robust tracking, detection and capture of rare cells.

8956-16, Session 4

Novel bio-imaging techniques using optical highlighter fluorescent proteins

Wei Min, Xinxin Zhu, Columbia Univ. (United States)

Fluorescence imaging of genetically encoded fluorescent proteins has transformed cell biology and neurobiology. Optical highlighters are a remarkable family of fluorescent proteins that could alter their excitation and emission spectra upon proper external light illumination. Since their first appearance about a decade ago, optical highlighters have played a major role in super-resolution imaging, protein dynamics, gene expression and cellular trafficking et al. Here we are harnessing optical highlighters to develop three distinct new bio-imaging techniques: genetically encoded microviscosity sensors using the newly discovered protein-flexibility mediated photochromism effect of Dronpa derivatives (Proc. Natl. Acad. Sci. USA, 2012), high-contrast deep tissue imaging with super-nonlinear fluorescence microscopy mediated by molecular switches (Optics Express, 2012; Biomedical Optics Express, 2012; J. Phys. Chem. Lett. 2012), and light-driven fluorescent timer for simultaneous spatial-temporal mapping of protein turnover dynamics in live cells. The unique on-off switch capability of these optical highlighter fluorescent proteins has been indispensable in the working principles of all these new bio-imaging techniques.

8956-17, Session 4

Initial formal toxicity evaluation of APC-2, a novel fluorescent tracer agent for real-time measurement of glomerular filtration rate in preparation for a first-in-man clinical trial

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The fluorescent tracer agent 2,5-bis[N-(1-carboxy-2-hydroxy)]carbamoyl-3,6-diaminopyrazine, designated APC-2, has been developed with properties and attributes necessary for use as a direct measure of glomerular filtration rate (GFR). Comparison to known standard exogenous GFR agents in animal models has demonstrated an excellent correlation. A clinical trial to demonstrate this same correlation in humans is in preparation. A battery of formal toxicity tests necessary for regulatory clearance to proceed with a clinical trial has been recently completed on this new fluorescent tracer agent. These include single dose toxicity studies in rats and dogs to determine overall toxicity and toxicokinetics of the compound. Blood compatibility, mutation assay, chromosomal aberration assay, and several other assays were also completed. Toxicity assessments were based on mortality, clinical signs, body weight, food consumption and anatomical pathology. Blood samples were collected to assess pharmacokinetic parameters including half-life, area under the curve, and clearance. Urine samples were collected to assess distribution. Doses of up to 200-300 times the estimated human dose were administered. No test-article related effects were noted on body weight, food consumption, ophthalmic observations and no abnormal pathology was seen in either macroscopic or microscopic evaluations of any organs or tissues. All animals survived to scheduled sacrifice. Transient discoloration of skin and urine was noted at the higher dose levels in both species as expected from a highly fluorescent compound and was not considered pathological. Thus initial toxicology studies of this new fluorescent tracer agent APC-2 have resulted in no demonstrable pathological test article concerns.

8956-18, Session 4

Microencapsulation of curcumin in PLGA microcapsules by coaxial flow focusing

Fan Lei, Ting Si, Xishen Luo, Univ. of Science and Technology of China (China); Ronald X. Xu, The Ohio State Univ. (United States)

Curcumin, a natural polyphenolic compound, has shown promising chemopreventive and chemotherapeutic activities in cancer. However, Although phase I clinical trials have shown curcumin as a safe drug even at high doses, poor bioavailability and suboptimal pharmacokinetics largely moderated its anti-cancer activity in pre-clinical and clinical models. To improve its applicability in cancer therapy, we report a liquid-driven coaxial flow focusing process to encapsulate curcumin in poly(lactic-co-glycolide) (PLGA) microcapsules. In the process a coaxial cone-jet configuration can be formed in the core of a high-speed coflowing liquid flow and the coaxial liquid jet eventually breaks up into

microcapsules because of flow instability. In experiments, the process setup consists of a customized coaxial needle, a two-channel and a high pressure syringe infusion pump, a particle collection reservoir and a process monitoring system. PLGA dissolved in dichlormethane and curcumin dissolved in acetone are supplied by the two-channel syringe infusion pump through outer and inner of the coaxial needle. The coaxial needle is fixed on a container facing a small hole located on the bottom of the container. Water with surfactant is injected into the container through a hole by the high pressure syringe infusion pump. Confocal and SEM images are used to show the size distribution and core-shell structure of the microcapsules. The drug loading is measured and an in vitro release simulation is carried out to record the release profile of these microcapsules. The results are further compared with those prepared by commonly used double emulsion process.

8956-43, Session 4

Novel copper quenched fluorescent activatable molecular probes

Dolonchampa Maji, Mingzhou Zhou, Washington Univ. School of Medicine in St. Louis (United States); Pinaki Sarder, Washington Univ. in St. Louis (United States); Samuel Achilefu, Washington Univ. School of Medicine in St. Louis (United States)

No Abstract Available

8956-19, Session 5

Quantifying the surface chemistry of 3D matrices in situ

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The insoluble microenvironment of cells (matrix) is a major modulator of cell phenotypes. However, at the moment there are very few methods for quantifying the physicochemical properties of a 3D matrix in situ. This study describes a novel imaging-based method for quantifying in situ the surface chemistry (ligands for particular cell adhesion receptors) of 3D matrices. The method is an in situ binding assay that utilizes fluorescently labelled recombinant proteins (markers) that emulate the binding properties of the adhesion receptor of interest. Binding of fluorescent markers on their ligands on the 3D matrix is detected via multi-photon microscopy. The method is applied in quantifying the density of adhesion ligands for the two major collagen-binding integrins (?1?1, ?2?1) on the surface of two kinds of porous collagen biomaterials. One ("active") is similar to biomaterials used clinically to induce regeneration in injured skin and peripheral nerves. The second ("inactive") has been shown experimentally to induce poor regeneration in animals. The binding properties of the markers are validated by biochemical assays. Results provide the first in situ quantification of the surface chemistry (density of ?1?1, ?2?1 adhesion ligands) of a biomaterial and demonstrate that chemical cross-linking can deplete collagen scaffolds of CBI ligands, therefore can change completely the way cells sense and respond to them. The proposed methodology can be utilized to quantify both biomaterials and decellularized extra-cellular matrix (ECM) of tissues in physiological or pathological conditions.

8956-20, Session 5

In vivo molecular mapping of an AOM-treated mouse model of colon carcinogenesis

Sarah J. Leung, Photini F Rice, Univ. of Arizona (United States); Jennifer K. Barton, Univ .of Arizona (United States)

Development of molecular probes and imaging strategies enables improved and novel studies of animal disease models, which may then lead to more effective diagnostic and treatment strategies for humans in the clinic. Here we introduce the use of a miniaturized multimodal endoscope equipped with optical coherence tomography (OCT) and laser induced fluorescence (LIF) imaging modalities with lavage-delivered antibodies conjugated to fluorescent Cy5.5 to create a time-resolved molecular map of colon carcinogenesis in an azoxymethane (AOM)–treated mouse model. The endoscopic imaging system enables non-destructive, high resolution and high sensitivity imaging of the distal colon of AOM-treated mice. OCT provides information on the gross structural tissue changes. LIF, in combination with synthesized fluorescent contrast agents optimized for low signal background with tissue and the multimodal endoscope, provides sensitivity to shifts in the expression of molecular markers. We monitored in vivo changes in expression of epithelial growth factor receptor (EGFR), transferrin receptor (TfR), transforming growth factor beta (TGF?), and chemokine (C-X-C motif) receptor 2 (CXCR2) over a five month period in mice following AOM administration. In vivo OCT and LIF imaging was validated against frozen histological sections of colon tissue collected after mouse sacrifice. This combined system enabled time-serial imaging of an individual animal's disease progression and we were able to correlate fluctuating expression of monitored markers with the development of adenocarcinoma. Using this strategy to track molecular expression, we may be capable of earlier detection, minimally invasive staging, and effective therapeutic targeting of colorectal cancer.

8956-21, Session 5

Reevaluation of biotin-streptavidin conjugation in fret applications: buffer solution strongly influences the transfer efficiency

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Often adopted in imaging and sensing systems, the Förster resonance energy transfer (FRET)-based systems utilizing dye-labeled biotinylated-DNA (DLB-DNA) molecules and streptavidin-coated quantum dots (SA-QDs) convey the obvious advantage of the capability of precise distance control between the QD and dye by varying the number of DNA base or dye positions on the DNA chain. The dependence of FRET efficiency on the distance has been manifested by means of monitoring fluorescence intensity change, whereas independence was reported recently (1). To address this controversial issue, we herein investigated the FRET efficiency in different buffer solutions through fluorescence lifetime measurements. Single-stranded DNA was labeled with biotin at 3' end and Alexa Fluor-750 dye at 5' end. The samples were mixed with SA-QDs (emmax: 655 nm) in an incubation buffer (I-buffer) and then transferred into a dilution buffer (D-buffer) prior to measurement. Our results suggest that FRET efficiency is strongly influenced by the salt content of buffer. When relatively high ionic-strength buffer (i.e. PBS) was employed as I-buffer, FRET efficiency was surprisingly negligible; when PBS as D-buffer, the FRET efficiency was found remarkably high but not dependent in DNA length. However, a good dependence was obtained when low ionic-strength buffers were used, such as 10 mM Tris buffer, regardless as I-buffer or D-buffer. Such dependence disappeared when salt was added into the tested samples. Our findings indicate that the DLB-DNA molecule might become randomly-coiled in high ionic-strength buffer, which is unbeneficial for the binding to SA-QDs in initial incubation stage (cause no FRET) or makes the distance control retarded in the second stage of dilution by shortening the length or bending toward SA-QDs surface (cause no dependence).

Reference.

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8956-22, Session 5

Detection of colorectal cancer using nir quantum dots as contrast agents in a mouse model

Jordan L. Carbary, Jennifer Barton, Urs Utzinger, The Univ. of Arizona (United States)

Optical Coherence Tomography/Laser Induced Fluorescence (OCT/LIF) dual-modality imaging has been shown to provide more information on colorectal cancer imaging in mice than either one alone. Ideally, the contrast agent used for this system should not compete with autofluorescence of the tissue or with the OCT wavelength range. NIR quantum dots (QD) have great potential as contrast agents for this dual-modality approach. As a preliminary ex vivo investigation into their efficacy, commercially available and in-house made NIR quantum dots (655nm, 940nm emission) targeted to vascular epithelial growth factor receptor 2 (VEGFR2), which has been shown to be upregulated in colon neoplasms, were applied in vivo to the colon of carcinogen or saline treated mice and allowed to incubate. At the end of the incubation, the colon was explanted and imaged using wide-field fluorescence microscopy for an en face image. OCT images were also obtained prior to colon labeling for comparison of tumor location. This presentation will include contrast analysis on the images obtained from the wide-field fluorescence microscopy, as well as comparisons of tumor locations between the fluorescence and OCT images. The contrast analysis provides the ability to elucidate the efficacy of the QDs as contrast agents for an in vivo pre-clinical study, whereas the OCT/fluorescence image comparisons begin to provide evidence of the ability to increase detection efficacy when using dual-modality imaging.

8956-23, Session 5

Measurement of absolute fluorescence quantum yields in the near-infrared and infrared spectral region

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There is an increasing interest in molecular dyes, nanoparticles, and nanocrystalline materials with emission in the near-infrared (NIR) and infrared (IR) region for applications as fluorescent reporters in biomedical imaging and as active components in electrooptical devices [1,2]. At the core of the assessment and comparison of material performance and the development of rational design strategies for improved systems are spectroscopic tools for the determination of the signal-relevant optical properties of these materials like their fluorescence quantum yields and brightness values [3-5].

This encouraged us to built up an integrating sphere setup for absolute measurements of fluorescence quantum yields of liquid and solid, transparent and scattering materials in the wavelength region from 700 nm to 1600 nm in addition to our setup for measurements in the wavelength region from 350 nm to 1000 nm. In addition, a setup was developed for excitation power density dependent measurements of upconversion fluorescence quantum yields. Here, we discuss the design strategies for such a setup and its characterization and present absolute fluorescence quantum yields of organic dyes and NIR- and IR-emissive quantum dots such as differently sized PbS and CdHgTe of different size and material composition.

8956-24, Session 6

NIR to NIR upconversion in KYb2F7: RE3+ (RE = Tm, Er) nanoparticles for biological imaging

Francisco J. Pedraza, Brian G. Yust, Annette Rodriguez, Andrew Tsin, Colleen Witt, Dhiraj K. Sardar, Univ. of Texas at San Antonio (United States)

Until recently, many contrast agents widely used in biological imaging have absorbed and emitted in the visible region, limiting their usefulness for deeper tissue imaging. In order to push the boundaries of deep tissue imaging with non-ionizing radiation, contrast agents in the near infrared (NIR) regime, which is not strongly absorbed or scattered by most tissues, are being sought after. Upconverting nanoparticles (UCNPs) are attractive candidates since their upconversion emission is tunable with a very narrow bandwidth and they do not photobleach or blink. The upconversion produced by the nanoparticles can be tailored for NIR to NIR by carefully choosing the lanthanide dopants and dopant ratios such as KYb2F7: RE3+ (RE = Tm, Er). Spectroscopic characterization was done by analyzing absorption, fluorescence, and quantum yield data. In order to study the toxicity of the nanoparticles Monkey Retinal Endothelial Cells (MREC) were cultivated in 24 well plates and then treated with nanoparticles at different concentrations in triplicate to obtain the optimal concentration for in vivo experiments. It will be shown that these UCNPs do not elicit a strong toxic response such as quantum dots and some noble metal nanoparticles. 3-D optical slices of nanoparticle treated fibroblast cells were imaged using a confocal microscope where the nucleus and cytoplasm were stained with DAPI and Alexa Fluor respectively. These results presented support the initial assumption, which suggests that KYb2F7: RE3+ would be excellent candidates for NIR contrast agents.

8956-25, Session 6

Synthesis of biocompatible SiO2 coating on luminescent nanoparticles of ZrO2:Er-Yb and Y2O3:Er-Yb

Tzarara Lopez Luke, Ctr de Investigaciones en Óptica AC (Mexico); Andrea Martinez, Centro Univ. de los Lagos (Mexico); Andrea Ceja, Ctr. de Investigaciones en Óptica AC (Mexico); Ruben Rodriguez, Centro Univ. de los Lagos (Mexico); Ana Lilia Gonzalez Yebra, Beariz Gonzalez Yebra, Univ. de Guanajuato (Mexico); Pedro Salas, Univ. Nacional Autónoma de México (Mexico) and Ctr. de Fisica Aplicaday Tecnologia Avanzada (Mexico); Elder De La Rosa Cruz, Ctr. de Investigaciones en Óptica AC (Mexico)

Recently, upconversion fluorescence from lanthanide-doped nanocrystals excited with infrared light has attracted much attention due to the potential applications as sensitive biolabelings, as improved alternatives to fluorophores and Quantum Dots (QD's). Zirconium and Yttrium oxide are an excellent materials in photonic applications due to their optical properties and low phonon energy, also can easily be doped with rare earth due to their structure. In this work, such oxide nanocrystals were doped with ytterbium and erbium 2 to 1 % mole respectively. The size of Zirconium and Yttrium oxide nanoparticles synthetized were of 20 and 70 nm respectively. Oxide nanoparticles were coated with a thin layer of silica and then functionalized with a layer of biotin to which is anchored the anti Ki67 protein. The conjuagated nanoparticles were used to detect cervix cancer in vitro. The different layers were studied by FTIR and Raman spectroscopy. The silica shell can prevent possible toxic effects from the nanoparticles and have a surface that facilitates the conjugation with biomolecules. The main advantages to use Er-Yb doped Y2O3 and ZrO2 with visible emission is that the excitation with Infrared light is not absorbed by tissue avoiding the self-fluorescence.

8956-26, Session 6

(Bio)hybdrid materials based on optically active nanoparticles

Manuela Reitzig, Juliana P. L. Goncalves, Fraunhofer IZFP-D (Germany); Afnan Q. Shaikh, Fraunhofer IZFP-D (Germany) and Max Bergmann Ctr. of Biomaterials (Germany); Daria Kovalenko, Susan Derenko, Thomas Härtling, Jörg Opitz, Fraunhofer IZFP-D (Germany)

In this contribution we provide an overview over our current investigations on optically active nanoparticles (nanodiamonds, upconversion phospors, metal particles) for biohybrid and sensing applications.

Due to their outstanding properties nanodiamonds gain attention in various application fields such as microelectronics, optical monitoring, medicine, and biotechnology. Besides the typical diamond properties such as high thermal conductivity and extreme hardness the carbon surface and its various functional groups enable different chemical and biological surface modification. At Fraunhofer IZFP we modify (bio) surfaces by integrating chemically modified nanodiamonds. This strategy is applied for very diverse surfaces ranging from bone implants to steel pipelines. For the first purpose, nanodiamonds are chemically modified at their surface with amino or phosphonic functionalities that are known to increase adhesion to bone or titanium alloys, while in the second case mechanical implementation into a coating is pursued.

In the contribution we furthermore highlight how another class of nanomaterials, namely upconversion phosphors, are used to verify sterilization processes. We found that these materials change their optical properties when exposed to a sterilizing electron beam. Implementing upconversion phosphors into packaging materials therefore enables an indirect verification of surface sterilization.

Last but not least we will review our recent investigations of metal nanoparticle arrays and their implementation into sensor systems. Especially gold particles can specifically be modified at the surface to address certain target molecules. Specific binding of analytes leads to a shift of the plasmonic resonance peak which can be used for detection of e.g. biomolecules in water.

8956-27, Session 7

Ultrabright and bleaching-resistant hybrid gold nanoparticles for confocal and two-photon fluorecence imaging

Patrice L. Baldeck, Univ. Joseph Fourier (France)

No Abstract Available

8956-28, Session 8

Use of fluorescent NIR dyes in silica nanoparticles and as enzyme substrates in bioanalytical applications *(Invited Paper)*

Gabor Patonay, Maged Henary, Gala Chapman, Garfield Beckford, Georgia State Univ. (United States); Holly Ellis, Auburn Univ. (United States)

Near-Infrared (NIR) absorbing carbocyanine dyes have been increasingly used in analytical, biological and medical fields as they can be useful for developing bioanalytical and biomedical methods. NIR dyes typically have relatively low fluorescent quantum yield as compared to visible fluorophores, but much higher molar absorptivities. Fluorescence intensity of NIR dyes significantly increases by enclosing several dye molecules in silica nanoparticles. Self quenching may become a problem for carbocyanines at high concentration in the silica nanoparticle. 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 also serves as a linker to covalently attach the dye molecule to the nanoparticle backbone. The primary applications of these particles are for bright fluorescent labels to be used in bioanalytical applications such as immunochemistry, flow cytometry, etc. Carbocyanines containing alkylsulfonate moieties do not exhibit significant fluorescence change upon binding to biomolecules however otherwise identical NIR dyes that contain alkylaldehyde moiety at the same position do exhibit changes which can be used for characterization of alkenesulfonate monooxygenase enzyme activity using near infrared dyes as substrates. In this study a new class sulfonated penta- and heptamethine dyes using in vitro photo-reduced riboflavin mononucleotide (FMN) with a glucose/ glucose-oxygenase oxygen scavenging system were used. Laser Induced Fluorescence (LIF) detected CZE was utilized to detect the sulfonated and de-sulfonated carbocyanines. The lower fluorescence quantum yield of the less water soluble alkylaldehyde analogs was detected and enzyme activity was characterized.

8956-29, Session 8

Optical nucleic acid switches for sensing in cell

Ambra Giannetti, Sara Tombelli, Cosimo Trono, Istituto di Fisica Applicata Nello Carrara (Italy); Barbara Adinolfi, Paola Nieri, Univ. di Pisa (Italy); Greta Varchi, Istituto per la Sintesi Organica e la Fotoreattività (Italy); Francesco Baldini, Istituto di Fisica Applicata Nello Carrara (Italy)

Nanoparticle and nanomaterial technologies in the biomedical field are significantly impacting the development of both therapeutic and diagnostic agents. The equipment of complex nanostructures with optical nanoprobes, capable of achieving quantitative information on intracellular events is further increasing the potential impact of these tools. It was shown that oligonucleotide optical switches could play a fundamental role in this area, since they can be used not only as simple on-off elements but also as real sensors. We will describe different types of optical nucleic acid switches focusing on their use as intracellular nanosensors: oligonucleotide optical switches are among the most promising optical nanosensors proposed in the recent years. They are suitable molecules capable to turn on or to modify their light emission upon the molecular interaction with well-defined molecular targets. Among these types of probes, molecular beacons (MBs), or hairpin DNA flares/probes, have been used in a variety of applications, including intracellular sensing.

We will also describe the design, implementation and characterization of structured polymethylmethacrylate (PMMA) nanoparticles conjugated to a specific molecular beacon for intracellular mRNA monitoring.

8956-30, Session 8

Effects of ICG concentration and particle diameter on photophysical properties of ICG-doped nanoparticles

Jason Crovisier, Baharak Bahmani, Reema Saleh, Valentine Vullev, Bahman Anvari, Univ. of California, Riverside (United States)

The variety of nanoparticles developed by numerous investigators has presented a diverse platform for various optical imaging applications in biomedicine. We have previously reported that the FDA-approved chromophore Indocyanine Green (ICG) can be successfully encapsulated by cross-linked poly-allylamine hydrochloride (PAH)-Disodium

Monophosphate (Na2HPO4) to form a nanoparticle for near-infrared imaging applications. The diameter of the constructs are dependent on the charge ratio between the polymer and salt used to encapsulate the chromophore. Modifications of the synthesis methods can alter the photophysical properties of the capsules, either through the adjustment of the charge ratio between PAH and Na2HPO4 or concentration of ICG successfully impregnated into the capsule. Through understanding the effects of tuning the nanoparticle properties, the photophysical characteristics of the constructs can be optimized. Here we present the results of adjusting the diameter of the nanoparticle and the concentration of ICG on the relative fluorescence quantum yield. Optimizing the photophysical properties can lead to increased imaging sensitivity and contrast for potential translational applications, including tumor imaging, which may utilize these nanoconstructs.

8956-31, Session 8

Heterogeneous nanostructures for plasmonic interaction with luminescence and quantitative surface-enhanced Raman spectroscopy

Gautom K. Das, Sudheendra Laskhmana, Ian M. Kennedy, Univ. of California, Davis (United States)

NIR-to-visible up-conversion nanomaterials have been investigated in many promising applications including next-generation displays, solar cells, and biological labels. When doped with different trivalent lanthanide ions, NaYF4 nanoparticles can be produce up-converted emission from infra-red excitation to visible wavelength emission. However, the quantum yield of this class of materials is low. Noble metals in the vicinity of the phosphor can increase the fluorescence by local field enhancement due to plasmonic resonances, and by modification of the radiative rate(s) of the phosphor. Most previous studies investigated the phenomenon by placing nanophosphors on to a metal substrate, or by embedding phosphors in glass, or by fabrication of core/shell structures with spacers such as polymers, dielectric materials (silica). However, fabrication of a uniform shell of noble materials onto up-converting phosphors in solution has been a challenge. We present a novel method for the fabrication of a uniform shell of silver onto lanthanide doped-NaYF4 nanophosphors. The thickness of the metal shell was systematically varied to show the proximity effect of metals with the phosphors. In addition, we have successfully applied this novel nanostructure to surface-enhanced Raman spectroscopy (SERS).

8956-32, Session 8

SERS substrates with star-like gold nanoparticles for sensing low concentration molecules

Elder De La Rosa Cruz, Leonardo Perez Mayen, Tzarara Lopez-Luke, Ctr. de Investigaciones en Óptica AC (Mexico); Andrea Ceja, Ctr de Investigaciones en Óptica AC (Mexico)

Surface enhanced Raman scattering (SERS) with metallic nanoparticles (NPs) is considered to be the best approach for sensing low concentration of molecules. Several techniques have been used for the preparation of such substrate but Langmuir Blodgett (L-B) methodology is an excellent solution considering the easy and cheaper process. The main problem is to guaranty repeatability. Morphology plays an important role because plasmon tunability. In this work, it is reported the use of star-like gold NPs with small peaks that can confine better the electric field and helps to enhance the detection limit. The lower concentration detected with substrate prepared with this kind of NPs is 10-18 M of Rhodamine B (RhB). The results obtained are reproducible and repeatable by different substrates made in the same conditions. Furthermore, it is demonstrated a strong enhancement of the signal by the interaction between NPs and metallic film on the substrate. The morphology effect is demonstrated by comparing the Raman signal for spherical, star-like and rod gold NPs. The processes for substrates preparation consist in tree steps. First, the preparation of NPs and functionalized hydrophobic silicon substrates; second, estimate the quantity of nanoparticles solution necessary for a single layer deposition and set up the L-B equipment; third, execute the deposition of nanoparticles onto the substrate. After substrate is ready, the characterization process begins, it consists on the examination of the substrate by Atomic Force Microscope, and testing the sample using it for enhances Raman signal from well-known molecule like Rodhamine B and glucose.

8956-33, Session 9

Rapid imaging of tiny tumors in resected human breast and lung tissues by topically applying a novel fluorescence probe for GGT *(Invited Paper)*

Yasuteru Urano, Univ. of Tokyo (Japan) and Graduate School of Medicine (Japan) and Japan Science and Technology Agency (Japan)

We have synthesized a series of rhodamine derivatives whose original carboxylate group was converted to a hydroxymethyl group, and found that the acetylated derivative of hydroxymethyl rhodamine green (Ac-HMRG) exists as a closed spirocyclic structure in aqueous solution at physiological pH, whereas HMRG itself takes an open non-spirocyclic structure. Based on these findings, we have established a general design strategy to develop highly sensitive fluorescence probes for proteases and glycosidases, by replacing the acetyl group of Ac-HMRG with a substrate moiety of the target enzyme. We designed and synthesized fluorescence probes for gamma-glutamyl transpeptidase (GGT), leucine aminopeptidase, fibroblast activation protein, beta-galactosidase, and so on. All these probes were almost non-fluorescent due to the formation of spirocyclic structure, but were converted efficiently to highly fluorescent HMRG by the target enzymes.

Some protease activities are well known to be upregulated in cancer cells. We have applied our novel protease-activatable probes to cancer bearing mice, and found that gGlu-HMRG, a novel probe for GGT, can visualize tiny tumor sites within a minute, even when they are less than 1 mm in size, by topically spraying onto tissue surfaces. Then, we started to examine the efficacy of gGlu-HMRG with freshly resected human tumor samples as an intraoperative tumor detecting agent. In this conference, we will discuss the results with resected human breast and lung cancer samples. We believe that the ease of spraying activatable fluorescence probes in open surgery or through catheters will provide alternative image guidance during treatment.

8956-34, Session 9

The role of the chromophore electronic structure and electrostatic interactions in photoconversion of red fluorescent proteins

Alexander Mikhaylov, Milkhail Drobizhev, Lauren Barnett, Geoffrey Wicks, Montana State Univ. (United States); Yuriy Stepanenko, Institute of Physical Chemistry of Polish Academy of Sciences (Poland); Thomas E. Hughes, Patrik R. Callis, Aleksander Rebane, Montana State Univ. (United States)

Fast photobleaching and photoconversion of red fluorescent proteins (RFPs) are serious factors in conventional imaging techniques, as well as in new supper-resolution micropscopy (PALM and STORM). One

needs therefore to understand the underlying molecular mechanisms. Recently we have shown that the modulation of the bond order in the chromophore bridge of the RFPs can efficiently control the rate of their reversible photoconversion. To get further insight, we have studied the temperature dependence of the photo-transformation dynamics in 12 RFP mutants and found that the observed rates can be described by a superposition of temperature-dependent and temperature-independent contributions of the similar magnitude. The temperature-independent contribution, of the order of 10^4 – 10^5 s^-1, is most probably due to a rotation around the imidazole- or phenyl-bridging bond. According to our hypothesis, this rotation is hindered by strong electrostatic interaction of an anionic chromophore with a nearby positively charged K163 residue (as in DsRed). The temperature independence reflects an almost barrierless rotation around this bond. The low frequency of attempts is probably due to the collective "breathing" motion of the protein, resulting in rare events of sudden and drastic decrease of the barrier when the bond order becomes close to single. The temperature-dependent contribution is described by an Arrhenius law with the frequency of attempts ~5x10^12 s^-1 and barrier energies in the range of 10 – 14 kcal/mole. This information helped us to create a more stable mutant of a popular RFP, mCherry.

8956-35, Session 9

A multidimensional screening method for the selection of two-photon enhanced fluorescent proteins

Caleb Stoltzfus, Lauren Barnett, Aleksander Rebane, Thomas Hughes, Mikhail Drobizhev, Geoffrey Wicks, Alexandr Mikhailov, Montana State Univ. (United States)

Two photon excitation of fluorescent proteins (FPs) is widely used in imaging whole organisms or living tissues. Many different FPs are now available but these proteins have only been optimized for their one photon properties. We have developed a technique for screening entire libraries of E. coli colonies expressing FPs that utilizes multiple wavelengths of linear excitation as well as two photon excitation. Single mutations in a particular protein that affect one or two photon properties are easily identified, providing new views of structure/function relationships. An amplified femtosecond Ti:sapphire laser and a spectrally filtered lamp source are used to acquire the fluorescence signals of up to ~1000 E. coli colonies on a standard Petri dish. Automation of the analysis and acquisition of the fluorescent signals makes it feasible to rapidly screen tens of thousands of colonies. In a proof of principle experiment with the commonly used eGFP, we used two rounds of error prone PCR and selection to evolve new proteins with shifted absorption and increased two-photon cross sections at 790nm. This method of screening, coupled with careful measurements of photo bleaching dynamics and two photon cross sections, should make it possible to optimize a wide variety of fluorescent proteins and biosensors for use in two photon microscopes.

8956-36, Session 9

The study of hydrogen peroxide level under cisplatin action using genetically encoded sensor hyper

Anna G. Orlova, Institute of Applied Physics (Russian Federation); Anna V. Maslennikova M.D., Institute of Applied Physics (Russian Federation) and Nizhny Novgorod State Medical Academy (Russian Federation); Anastasia S. Belova, N.I. Lobachevsky State Univ. of Nizhni Novgorod (Russian Federation) and Institute of Applied Physics (Russian Federation); Ekaterina A. Sergeeva M.D., Institute of Applied Physics (Russian Federation); Anna A. Brilkina, N.I. Lobachevsky State Univ. of Nizhni Novgorod (Russian Federation); Natalia M. Mishina, Nizhny Novgorod State Medical Academy (Russian Federation) and Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry (Russian Federation); Natalia M. Shakhova M.D., Institute of Applied Physics (Russian Federation) and Nizhny Novgorod State Medical Academy (Russian Federation)

Chemotherapy is one of the main methods of cancer treatment. It is well known that certain chemotherapeutical methods are based on cancer cells sensitivity to oxygen stress and reactive oxygen species play significant role in realization of cytotoxic action of antineoplastic agents. The goal of the study was the investigation of the level of hydrogen peroxide in tumor cells in vitro during exposing them with cisplatin. Cell line of human cervical carcinoma HeLa Kyoto, expressing the cytosolic sensor for hydrogen peroxide HyPer was used. Assessment of cisplatin cytotoxicity has been performed using MTT assay. Drug concentrations that cause 50% cell death (IC50) was chosen for the study of ?2?2 dynamics in cells. HyPer is a genetically encoded ratiometric sensor that responds to changing of ?2?2 concentration by changing of the ratio of fluorescence signals under the illumination at wavelengths of two excitation maxima of the sensor (420 nm and 500 nm). HyPer reaction has been estimated using Laser Scanning Microscopy (excitation at 458 nm and 488 nm; registration of fluorescence in the range of 500-530 nm). Cisplatin addition does not induce rapid changes of the ratio of fluorescence signals under excitation at 458 nm and 488 nm in the cytoplasm of tumor cells during the period of observation (30 min). It can be supposed that H2O2 does not take part in HeLa Kyoto early response on cisplatin exposure. It is necessary to identify the role of other reactive oxygen species in cisplatin-induced reaction in the further research.

8956-37, Session 9

Efficient delivery of antigen and near infrared dye to mature dendritic cells for tumor immunotherapy

Honglin Jin, Yuan Qian, Sha Qiao, Chuan Huang, Zhihong Zhang, Huazhong Univ. of Science and Technology (China)

Efficient and safe delivery of antigen and imaging agents to dendritic cells (DCs) are essential for DC-based immunotherapy. However, preparation of clinically usable DC vaccine requires immature DCs (imDCs) to be matured, which dramatically reduces their endocytic uptake abilities, and therefore restricting the delivery of exogenous cargo to mature DCs (mDCs). Here, we report an approach for simultaneous delivery of antigen and fluorophores to mDCs from mouse bone marrow by fabricating a biocompatible HDL-mimicking peptide-phospholipid scaffold (HPPS) nanocarrier. The near-infrared dye (DiR-BOA) was loaded into the hydrophobic core of HPPS, and glycoprotein 100 (gp 100) melanoma peptides were fused into the apoA-1 biomimetic peptides, resulting in a dual-functional lipid nanoparticle (DLP) with a diameter of 30 nm. Results show that over 99% of imDCs and mDCs were fluorescently labeled by DLP within 3 h incubation. The uptake of gp 100 peptide delivered by DLP in mDCs was 1.5-2 folds of that in imDC. Compared to gp 100 peptide, approximately 10-fold increased uptake in mDCs was found by using DLP. Confocal imaging studies demonstrated the uptake of DLP was through scavenger receptor B1 and gp 100 peptide was mainly localized in the cytosol. Moreover, we were able to track DLP-labeled mDC injected into the footpad into popliteal lymph nodes using NIR fluorescence. Mice immunized with mDCs containing DLP showed enhanced antigen specific T-cell responses and delayed tumor growth than controls. Together, this study provided a useful approach for efficient delivery of imaging agents and peptide antigen to mDC for tumor immunotherapy.

8957-1, Session 1

Low-cost, high-sensitivity SERS nano-bio-chip for kinase profiling, drug monitoring and environmental detection: a translational platform technology

Yi Chen, Logan Liu, Univ. of Illinois at Urbana-Champaign (United States)

The interaction of biomolecules and solid-state nanomaterials at the bio-nano interfaces is a long-lasting research topic in nanotechnology. Historically, fundamental problems, such as the electron transfer, energy transfer, and plasmonic interaction at the bio-nano interfaces, have been intensively studied, and revolutionary technologies, such as molecular electronics, peptide chips, nanoplasmonic sensors, have been created. With the combined effort of molecular dynamics simulation and surface-enhanced Raman spectroscopy, we studied the external electric field-induced conformation changes of dodecapeptide probes tethered to a nanostructured metallic surface. Through this study, we demonstrated a reversible manipulation of the biomolecule conformations as well as an in situ eletro-optical detection of the sub-nanometer conformational changes at the bio-nano interfaces. Based on the proof-of-concept established in this study, we further propose a novel nanophotonic peptide phosphorylation sensor for high-sensitive peptide kinase profiling. We have also demonstrated the same SERS nano-bio-chip can be used for environmental monitoring applications, such as detection of contaminants in drinking water at ultralow concentrates. The fabrication of this nanosensor is based on a single step, lithography-less nanomanufacturing process, which can produce hundreds of these chips in several minutes with nearly 100% yield and uniformity. Therefore, the demonstrated research can be readily translated into industrial mass productions.

8957-2, Session 1

Plasmonic gold nanostar probe for pH monitoring

Yang Liu, Hsiangkuo Yuan, Duke Univ. (United States); Andrew M Fales, Duke University (United States); Tuan Vo-Dinh, Duke Univ. (United States)

Cancer has become one of most significant death reasons and causes approximately 7.9 million human deaths worldwide each year. The challenge to detect cancer at an early stage makes cancer-related biomarkers sensing attract more and more research interest and efforts. Local pH environment has been identified to be a potential physiological biomarker for cancer diagnosis since cancer contains highly acidic environments. Surface-enhanced Raman scattering (SERS) provides a promising method for various biomarkers (DNA, RNA, protein, et al.) detection due to its high sensitivity, specificity and capability for multiple analyte detection. Raman spectroscopy is a non-destructive photon-scattering technique, which provides molecule-specific information on molecular vibrational energy levels. SERS takes advantage of plasmonic effects and can enhance Raman dramatically. A near-infrared (NIR) SERS nanoprobe based on gold nanostars for pH sensing is developed for single-cell studies and for use in cancer diagnostics. Near-infrared (NIR) light is suitable for in vivo applications because of its low attenuation rate and tissue autofluorescence. The changes in the SERS spectrum of a pH-sensitive reporter dye under various pH environments are monitored and used for pH sensing. Furthermore, density functional theory (DFT) calculation is performed to investigate Raman spectra changes with pH at the molecular level. The study demonstrates that SERS is a sensitive tool to monitor minor molecular structural changes due to local pH environment for disease detection.

8957-3, Session 1

Biosensing the progression of Huntington's disease using SERS

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As the elderly population increases worldwide, neurodegenerative disorders (ND) such as Parkinson's disease, Alzheimer's disease or Huntington's disease (HD) have become a growing challenge for healthcare systems leading to biomedical research focussed on detection and diagnosis as well as treatment.

Numerous NDs share the characteristic of atypical protein aggregations inside neuronal cells leading to cell death and the development of typical patient symptoms. HD is caused by a mutation in the huntingtin gene causing the expression and aggregation of mutant huntingtin protein (HTT). [1]

We have recently shown that functionalized gold nanoparticles can be used to report the chemical environment inside cells using surface-enhanced Raman spectroscopy (SERS). [2] Here we employ a similar strategy of using SERS nanoprobes for monitoring the progression of HD in homogenised brain samples from the cortex and striatum of transgenetic R6/2 mice which are commonly used as a model in HD.[3] R6/2 mice develop intranuclear inclusions of HTT in their neurons. [4] We are able to track and classify the development of HTT protein aggregates in samples of different stages of HD. Insights into the development of HTT protein inclusions in transgenetic R6/2 mice will help to understand the progression of this disease over time. Furthermore, our results show the potential to be translated to the clinic. The SERS based procedure developed in this study can be applied to body fluids such as blood or cerebrospinal fluid in patients in order to identify significant classification markers for the progression of this disease in patients.

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8957-4, Session 1

A biocompatible microneedle-based probe for in vivo intradermal surface-enhanced Raman scattering measurements

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In vivo characterization of intradermal components is important to clinical diagnosis. Among those techniques, e.g. magnetic resonance imaging and ultrasound imaging is capable of imaging below the skin. Recently, we realize the surface-enhanced Raman scattering (SERS) by using the

Ag-coated stainless steel microneedle probe to sensitively detect the chemical compositions and conformations of test molecules at a depth of more than 700 μm below a phantom that mimics the scattering and absorption of the human skin. Although this SERS strategy can eliminate the injection of SERS nanoparticles that can be toxic and allows simple needle administration, a problem could arise if these microneedles break inside our skin. Moreover, sharp injuries could also occur during needle disposal after usage.

In this study, we design and fabricate a biocompatible microneedle-based SERS probe for in vivo intradermal measurements. The fabricated hollow microneedle allows the laser light to propagate to a desired location at a depth of more than 700 μm below a skin phantom, and silver nanoparticles coated on the microneedle offers effective SERS augmentation for emitted Raman signals from the test molecules. In addition, the microneedle is comprised of a biocompatible material, in which the hardness of the microneedle could be easily modified after administration to prevent sharp injuries. These results demonstrate the potential of employing the microneedle based SERS probe for in vivo intradermal SERS measurements.

8957-5, Session 2

Plasmonic molecular sentinel nanoprobes for disease biomarker detection

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This presentation describes the development and applications of plasmonics and surface-enhanced Raman scattering (SERS) nanoprobes for use in disease biomarker detection. Plasmonics refers to the research area of enhanced electromagnetic properties of metallic nanostructures that produce ultrasensitive and selective detection technologies. We describe the development of unique metallic nanoprobes for SERS biosensing. The SERS technology can detect chemical and biological species with exquisite specificity due to the intrinsically narrow Raman peaks. A DNA-based technique incorporating surface-enhanced Raman gene probes, referred to as “Molecular Sentinels” (MS) can be used to detect gene targets (DNA, mRNA, or microRNA) via hybridization to nucleic acid sequences complementary to these probes. We have demonstrated the feasibility of multiplex detection using the SERS-based molecular sentinel (MS) technology in a homogenous solution. Recently, the MS biosensing modality has been further developed to a novel detection scheme and adapted to gold nanostars, a unique biocompatible nanoprobe platform that allows spectral tunability of the plasmonic absorption. The results of this study demonstrate the specificity and selectivity of the MS nanoprobes, as well as the ability to use MS nanoprobes for direct detection of disease biomarkers.

Acknowledgments. Supported in part by Defense Advanced Research Projects Agency (grant HR0011-13-2-0003) and the National Institutes of Health (grants R01 EB006201 and T32 EB001040). The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

8957-6, Session 2

Bifunctional nanoparticles for surface-enhanced Raman spectroscopy-based leukemia biomarker detection

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With the aging of the population of the western countries, acut myeloid leukemia (AML) incidence rates are rising. In Europe there are about 22250 newly diagnosed cases registered yearly that correspond to an incidence rate of 3.62/100000 with low geographical variation between regions.1 Applying the recently available therapies, a high proportion of the patients seems to reach complete remission according to microscopic, morphology based evaluations. However, these methods are not able to detect low amount of malignant cells, that are still able to proliferate and cause relapse, what is known as minimal residual disease (MRD) in AML patients. Reliable quantitative detection of MRD would allow the follow-up of the response to chemotherapy and the use of targeted, personalized treatments, what might be crucial in increasing the cure rate, especially in elderly patients.2 The expression level of the Wilm’s tumor (WT1) gene mRNA transcript determined by RT-PCR was shown to correlate with the tendency to relapse in AML patients. However, blood plasma WT1 levels are extremely low. Fast and sensitive quantification of the WT1 remains still a challenge for novel molecular diagnostic methods.3,4

We present here Surface Enhanced Raman Spectroscopic (SERS) based detection of WT1 sequence using dye labeled reporter oligonucleotide and magnetic core @ gold shell nanoparticles. Magnetic nanoparticles were synthesized by co-precipitation of Fe(II) and Fe (III) salts in aquous media. Gold shell was generated on the surface after modification with aminopropyltriethoxy silane and adsorption of gold nanoparticle seeds.5 Hydroquinone was applied as a reducing agent for the gold coating in water based reaction mixture at room temperature. Thiolated ssDNA complementers of the WT1 sequence were used to functionalize the gold shell with capture olinucleotides in a facile and fast two step method.6 Addition of other thiolated modifying compounds was also investigated in order to decrease aspecific binding of non-complementer DNA to the nanoparticles’ surface. The signal amplification performance of the core-shell colloidal SERS substrate was tested using malachite green as label dye. The Raman signal enhancing efficacy of the magnetic core @ gold shell nanomaterial was compared with the efficacy of spherical gold particles produced using the conventional citrate reduction method.7 The core-shell particles were found to be superior both regarding robustness is SERS and facile separation in a heterogeneous reaction system.

The good physicochemical characteristics of these particles and the sensitivity observed in SERS experiments allow us to expect good performance in the further development steps of a novel, fast and reliable spectroscopic method for WT1 detection in MRD patients.

8957-7, Session 2

Application of SERS spectroscopy for detection of trace components in urinary deposits

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Surface-enhanced Raman scattering (SERS) spectroscopy can be a useful tool in regard to disease diagnosis and prevention. Advantage of SERS over conventional Raman spectroscopy is its significantly increased signal (up to factor of 106-107) which allows detection of trace amounts of substances in the sample. So far, this technique is successfully used for analysis of food, pieces of art and various biochemical/biomedical samples. In this work, we survey the possibility of applying SERS spectroscopy for detection of trace components in urinary deposits. Early discovery together with the identification of the exact chemical composition of urinary sediments could be crucial for taking appropriate preventive measures that inhibit kidney stone formation or growth processes.

In this initial study, SERS spectra (excitation wavelength - 1064 nm) of main components of urinary deposits (calcium oxalate, uric acid, cystine, etc.) were recorded by using silver (Ag) colloid. Spectra of 10-4-10-5 M solutions were obtained. While no Raman signal was detected without the Ag colloid, characteristic peaks of the substances could be separated in the SERS spectra. This suggests that even small amounts of the components could be detected and taken into account while determining

the type of kidney stone forming in the urinary system.

We found for the first time that trace amounts of components constituting urinary deposits could be detected by SERS spectroscopy. In the future study, the analysis of centrifuged urine samples will be carried out. Ultimately, due to its simplicity, we hope to show the potential of SERS being used in routine analysis.

8957-8, Session 2

Biomolecular sensing for cancer diagnostics using highly reproducible SERS substrates

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Human cancer is a complex disease commonly induced by genetic instability and accumulation of multiple molecular alternations. As early stage diagnosis is relatively well correlated with improved survival rates, there is extreme interest in developing molecular detection technologies, which can screen for circulating biomarkers or cellular events indicative of a cancer state. Among different methods, Surface Enhanced Raman Scattering (SERS) is a uniquely useful signal transduction mechanism, allowing the characterization and detection of biomolecules due to its fingerprint-like nature and high sensitivity [1].

Here we report on an optimised class of SERS substrates, obtained by patterning gold with electron beam lithography [2], which unlocked the possibility to sense the sensing of glycerophosphoinositol (GroPIns) under physiological relevant condition. GroPIns is an abundant component of cell cytosol that regulates numerous pathways resulting in cell proliferation, differentiation and tumour invasion. High GroPIns levels have been reported in several tumour cells (such as thyroid cells transformed by Ras and RET-PTC oncogenes) and during the inflammatory response. In our experiments, we demonstrate that the minimum detectable GroPIns concentration is about 80 nM. Remarkably, this value is about two orders of magnitude lower than the minimum concentration expected for GroPIns in cells. We additionally show that the Au-fishnet SERS substrates can be used to predict accurately the GroPIns concentration even in a multicomponent mixtures using partial least square (PLS) regression analysis.

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8957-9, Session 3

Plasmonic spectra of individual subwavelength particles under the IR microscope: cells and airborne dust

James V. Coe, David B Lioi, Lindsey Shaffer, Marvin A Malone, Antriksh Luthra, Aruna Ravi, The Ohio State Univ. (United States)

A plasmonic metal film with a subwavelength hole array (a mesh) is used to capture an individual subwavelength particle, like a single yeast cell or airborne dust particle, and an imaging infrared (IR) microscope, records a scatter-free, IR absorption spectrum of the particle. Single particle spectra of wavelength scale particles usually suffer from large scattering effects. Work on single, live yeast cells, airborne particles from our laboratory air, and particles from the World Trade Center 9/11/01 event will be presented.

8957-10, Session 3

Application of innovative plasmonic nanostructures in bioanalytics

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Speed and easiness combined with high sensitivity as well as specificity are the key properties of modern analytical methods. Based on metal nanostructures which feature unique properties for advanced optical biosensing application, our research on a variety of innovative chip based techniques applying in bioanalytics will be introduced.

The excitation of localized and propagating surface plasmon polaritons of nanostructure metal surfaces leads to a strong enhanced electromagnetic field, which can be utilized to amplify the intrinsic weak Raman signals of molecules. This so called surface enhanced Raman spectroscopy (SERS) is of major interest for answering a broad range of bioanalytical questions due to its high sensitivity and fingerprint specificity. However, to establish SERS as a reliable routine detection method within this field, suitable and powerful SERS active substrates have to be developed. Within this contribution, a variety of fabrication strategies for the achievement of suitable SERS substrates are introduced as well as discussed in context to both drug monitoring in complex media (e.g. urine, blood) and the detection of low molecular weight substances like illegal dyes in food. To address these various application fields, we established different Lab-on-a-Chip-SERS (LOC-SERS) technologies combined with high sample throughput to the fast detection of drugs and ingredients. Especially, the advantageous droplet-based microfluidic platform has the potential to monitor drugs in human body fluids.

8957-11, Session 3

Fano-resonant mid-infrared metasurfaces: a new platform for bio-sensing and vibrational fingerprinting of proteins and cells

Gennady B. Shvets, Nihal Arju, Glen Kelp, Alexander B. Khanikaev, Albert Lee, Konstantin Sokolov, Institute for Fusion Studies, The Univ. of Texas at Austin (United States)

Metamaterials exhibiting Fano interference are emerging as a powerful platform for sensing minute amounts of materials, in some instances as small as a single molecular or atomic monolayer. The basic physical reason for that is their highly spectrally-selective response and very high optical intensity concentration near resonance that enable proximity sensing. This translates into order of magnitude higher signals compared with more traditional surface-enhanced infrared absorption measurements. Because optical resonances of meta-surfaces are determined by their sizes and geometries, they can be readily engineered to match various vibrational fingerprints of proteins, nucleic acids, and other biologically relevant entities. Non-specific bindings can be easily accounted for because of the extra specificity provided by tuning meta-surface resonances to vibrational fingerprints. By fabricating multiple plasmonic pixels, each tuned to different vibrational resonances, it is now possible to develop label-free high-throughput biosensors for monitoring the kinetics of antibody-antigen bindings. Recent experimental results demonstrating how such platform can be used to determine protein orientation on the surface [1] will be presented. Experimental results for several binding assays such as biotin/streptavidin monolayers, mono and bi-layers of binding proteins and peptide, and engineered peptides will be presented. Integration of meta-surfaces fabricated on

infrared-transparent substrates with microfluidic delivery systems will be demonstrated, and the prospect for parallel data acquisition using focal plane array (FPA) infrared detectors will be discussed.

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8957-13, Session 3

Application of lattice plasmon waves in a coupled bilayer plasmonic nanoantenna array for surface-enhanced Raman spectroscopy

S. Hamed Shams Mousavi, Farshid Ghasemi, Ali Asghar Eftekhar, Ali Adibi, Georgia Institute of Technology (United States)

Surface-enhanced Raman Spectroscopy (SERS) using lithographically fabricated or chemically synthesized nanoantennas is a well-established biomedical sensing technique. Chemically grown plasmonic nanoparticles provide excellent optical properties. However, the performance of the resulting SERS substrates is limited by the random orientation and distribution of the nanoparticles and their relatively small scattering cross-section. We present a coupled bilayer plasmonic nanoantenna array, fabricated using electron beam lithography (EBL). The structure is composed of an array of plasmonic nanodisks stacked on an array of nanoapertures, supported by dielectric nanopillars. The lateral coupling results in a propagative lattice plasmon mode, and the vertical coupling increases the local field enhancement. Through the combination of the increased cross-section, due to the lattice plasmon wave, and the higher confinement, due to vertical coupling between the two layers, the overall efficiency of the SERS substrate is greatly improved. The topology of the nanoantennas is tuned, such that a localized SPP resonance coincides with the lattice plasmon mode at its close-to-zero group velocity (Vg) region, resulting in the high field enhancement at the pump and emission frequencies, while containing the energy in a small region near the excitation area. To demonstrate the high sensitivity of our device, arrays of nanoantennas with different sizes and lattice constants were fabricated on an oxide wafer. A medium-sized protein, Streptavidin (53 kDa) with significant biomedical applications, was immobilized on the dielectric nanopillars. We show that spectroscopic sensing in low concentrations of the target molecule, e.g. 500 nM, is achievable using the optimally designed array.

8957-14, Session 4

Plasmonic improvement of microcavity biomedical sensor spectroscopic characteristics

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New opportunity to improve a sensetivity of a label-free biomolecule detection in sensing systems based on microcavity evanescent wave optical sensors has been recently found and is being under intensive development. Novel technique based on combination of optical resonance on microring structures with plasmon resonance. Recently developed tools based on neural network data processing can realize real-time identification of biological agents. So combining advantages of plasmon enhancing optical microcavity resonance with identification tools can give a new platform for ulta sensitive label-free biomedical sensor.

Our developed technique used standard glass and polymer microspheres as sensetive elements. They are fixed in the solution flow by adhesive layer on the surface being in the field of evanescence wave. Sensitive layer have been treated by gold nanoparticel (GN) solution. Another technique used thin film gold layers deposited on the substrate below adhesive. The light from a tuneable diode laser is coupled into the microsphere through a prism and was sharply focussed on the single microsphere. Images were recorded by CMOS camera. Normalized by free spectral range resonance shift of whispering gallery mode (WGM) and a relative efficiency of their excitation were used as input data for biomolecule classification.

Both biomolecules and NP injection was obtained caused WGM spectra modification. But after NP treatment spectral shift and intensity of WGM resonances in biomolecule solutions increased. WGM resonances in microspheres fixed on substrate with gold layer with optimized layer thickness in biomolecule solutions also had higher intensity and spectra modification then without gold layer.

8957-15, Session 4

Leaky surface plasmon waves in SPR sensing systems

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Sensors based on Surface Plasmon mode excitation have been studied extensively in the last decade. The early Kretschmann configuration and later the guided wave configuration have both been used to excite Surface Plasmon resonance at a metal and analyte interface for sensing essentially the refractive index of the analyte. More recently, enzymes are being immobilized on the metal interface to detect change in refractive index due the reaction of enzymes with glucose, urea, lipids etc. present in the analyte for use as bio-sensors. The thickness of the metal layer in both the configurations strongly affects the surface plasmon resonance. In the Kretschmann configuration, it is widely agreed that the reflection dip occurs at an angle, where nprismsin? equals the effective index of the surface wave supported by the metal-analyte interface. However, for a small metal thickness, less than the transverse decay ‘skin depth’, our investigation shows that the surface mode becomes a ‘leaky mode’ and the reflection minima corresponds to the excitation of this leaky mode. When a similar guided wave structure is used, maximum attenuation is also seen only for TM modes for which the effective indices match with the leaky surface plasmon mode. This observation is important when the configuration is used to quantify the refractive index of the analyte.

8957-16, Session 4

Multispectral imaging for high-throughput surface plasmon resonance biosensors

Alexandra Sereda, Lab. Charles Fabry, Institut d’Optique Graduate School (France) and HORIBA Scientific (France); Julien Moreau, Michael Canva, Lab. Charles Fabry, Institut d’Optique Graduate School (France); Emmanuel Maillart, HORIBA Scientific (France)

Surface plasmon resonance (SPR) sensing is a powerful tool for biomolecular interactions study, where real-time and label-free detection is of particular interest. To approach the theoretical resolution limit, most SPR-based systems have turned to either angular or spectral interrogation modes: by monitoring the plasmon dip shift, these methods offer very accurate measurements, but at the expense of high throughput screening.

For both techniques, the resolution is related to the experimental setup used to acquire the resonance profile as well as on the data processing performed to extract the plasmon dip position. In the case of spectral interrogation, the resonance profile can be obtained either by scanning the incident wavelength or using a spectrometer as the detector. While

the former maintains a 2D imaging capability at the expense of the measurement rate, the latter provides instantaneous spectral information, but for a limited portion of the biochip (1D-arrays). Therefore, combining spectral interrogation with an imaging system allowing high data throughput is still a challenge.

In this contribution, we present a detailed analysis of SPR multi-spectral imaging performances, both numerically and experimentally, with the aim of combining spectral interrogation with both real-time and 2D imaging capabilities. With only five interrogation wavelengths, this technique provides a resolution of 2*1E-6 RIU (or a minimum surface coverage detectable of around 1 pg/mm^2), which equals some of the best SPR imaging systems, but with a significant reduction in data dispersion and accurate measurements over a wider range of experimental conditions compared to the classical reflectivity interrogation mode.

8957-17, Session 4

Plasmonic nano-optical tweezers with in-situ sensing capability

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Plasmonic Nano-Optical Tweezers (PNOTs) offer the possibility of trapping objects beyond diffraction limit. Clearly high performance PNOTs with strong trapping force and large trapping volume will lead to many immediate applications in nanotechnology and life sciences. We have studied various kinds of PNOTs from the viewpoints of geometric parameters in relation to trapping performance. In particular, while the so-called “trap-and-sense” strategy remains largely unexplored, we first demonstrate that gold nano-island substrates (Au-NIS) formed from a controlled thermal annealing process may act as PNOTs with high degree of reproducibility. Since the excitation of highly localized field is within the near-field boundary of the PNOTs, we have exploited their application for surface enhanced Raman scattering (SERS) studies. Through simulation and experimental investigations, we show that an optimized Au-NIS exhibits strong trapping force when excited by photons with wavelengths near the localized surface plasmon (LSP) resonance peak. The trapping targets were photo-chemically synthesized silver nano-decahedrons (Ag-NDs) surface functionalized with 4-mercaptobenzoic acid (4-MBA) which acts as the Raman tag. After adjusting the LSP peak of the Au-NIS to 633nm (He-Ne laser line) by carefully tuning thestarting gold film thickness and annealing time, the substrate was found to be very effective for the generation of PNOTs. On this sample, we added a drop of solution containing 4-MBA functionalized Ag-NDs, steadily the Ag-NDs were immobilized by the PNOTs generated under He-Ne laser illumination. So the Raman signal of 4-MBA was detected by a Raman excitation laser at 785nm focused on the Au-NIS. In addition, the Au-NIS may also be fabricated on a fiber tip for increased device versatility. SERS-based bio-detection using PNOTs in Au-NIS has been experimentally verified. This “trap-and-sense” approach offers a convenient platform for achieving cost-effective large area PNOTs without the need of nano-lithography. Moreover, the proposed approach leads to the possibility of performing highly sensitive bio-detection in a dynamic environment where the sample is in a continuous flow state.

8957-18, Session 4

Three-dimensional metallic nanostructures for bulk and bio SPR sensing applications

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Surface Plasmon Resonance (SPR) sensing is one of the most common applications of metallic nanostructures and has been widely used for chemical detection, including bio-molecule sensing. The SPR sensing principle is based on the change in optical resonance position due to a change in refractive index at the nanostructure surface. Both the resonance shift per refractive index unit (RIU) and the resonance bandwidth determine the resolution of the SPR sensor. Many studies have investigated methods, such as alteration of the nanostructure feature shape and arrangement, in an attempt to improve the detection limit and the SPR sensitivity of metallic nanostructures.

In this paper, we introduce a 3D metallic nanostructure, which has higher SPR sensitivity (resonance shift per RIU) and narrower bandwidth compared to a conventional metallic nanohole array structure. Three dimensional nanostructures were fabricated with various geometrical parameters using electron beam lithography followed by a wet-etching fabrication process. Each device was optically characterized by measuring the transmission resonance properties in terms of resonance position, resonance bandwidth and bulk-SPR sensitivity. Experimental results were compared to FDTD simulations. Simulations showed that transmission resonances of the 3D nanostructures had optical properties related to localized SP and SP polariton coupling effects, which resulted in hot spots of high electric field intensity. Measurements of the SPR sensitivity of surface-functionalized (biotinylated thiol) 3D nanostructures indicated that the device has the ability to detect streptavidin and may be useful as a bio-SPR sensor.

8957-19, Session 4

DNA sensing with dynamic plasmonic antennas

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The acute distance sensitivity of plasmon coupling has allowed the development of colorimetric sensors and nanometre-sized rulers. In order to design a gold nanostructure with a reversible optical response sensitive to one DNA strand, we assemble particle dimers around a single dynamic DNA template exhibiting a specific recognition site.

Electrophoresis is used to produce purified suspensions of gold particle dimers linked by a single DNA template, with particle diameters ranging from 8 nm to 40nm and sample purities as high as 90%. The molecular template includes a 44-base stem-loop in order to switch its shape reversibly when binding to a target DNA strand. For 8nm diameter particle dimers, surface-to-surface distances are estimated in cryo-electron microscopy and indicate a reversible change of the surface-to-surface distance by a factor of 3. The reversible shift between the two states was demonstrated for a 30-minute reaction at room temperature several times in a row (L. Lermusiaux et al, ACS Nano 6, 10992, 2012).

In order to translate the dynamic switching of a single DNA scaffold in a measurable optical signal, we study the scattering cross-section and the plasmon coupling of single 40 nm diameter gold particle dimers by confocal scattering spectroscopy. Plasmon frequency change was demonstrated for several interparticle distances, using different DNA lengths (M. P. Busson et al, Nano Lett. 11, 5060, 2011). Preliminary results of optical properties of dynamic single groupings, indicate the possibility of DNA sensing at the single molecule level.

8957-20, Session 4

DNA-aptamer optical biosensors based on a LPG-SPR optical fibre platform for point-of-care diagnostic

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Surface Plasmon Resonance (SPR) is the base for some of the most sensitive label free optical fibre biosensors. However, most solutions presented to date require the use of fragile fibre optic structure such as adiabatic tapers or side polished fibres. On the other hand, long-period gratings (LPG) present themselves as an interesting solution to attain an evanescent wave refractive index sensor platform while preserving the optical fibre integrity. The combination of these two approaches constitute a powerful platform that can potentially attain the highest sensitivities as it was recently demonstrated by detailed theoretical study [1,2].

In this work, an LPG-SPR platform is explored in different configurations (metal coating over the LPG, after the LPG and between two LPG) operating in the telecom band (around 1550nm). For this purpose LPGs fabricated by the electric arc technique, with periods in the 300 to 500?m range are combined with tailor made metallic thin films. In particular, the sensing regions were coated with 2nm of chrome to improve the adhesion of a 20nm thick gold film followed by a 100nm thick layer of TiO2 dielectric material strategically chosen to attain plasmonic resonance in the desired wavelength range.

The obtained refractometric platforms were then validated as a biosensor. For this purpose the detection of thrombin using an aptamer based probe was used as a model system for protein detection. The surface of the sensing fibres was cleaned with ethanol and dried with N2 and the aminated thrombin aptamer (GGTTGGTGTGGTTGG) was immobilize by physisorption using Poly-L-Lysine (PLL) as cationic polymer. Preliminary results indicate the viability of the LPG-SPR-APTMER as a flexible platforms point of care diagnostic biosensors.

8957-21, Session 5

Indium-based nanoparticles as plasmonic enhancers in the UV region

Joanie Gagnon, François Magnan, Frederic-Georges Fontaine, Denis Boudreau, Univ. Laval (Canada)

Up until recently, most developments in the use of metal nanoparticles to enhance molecular fluorescence have been focused on plasmonic nanostructures based on silver and gold. Unfortunately, these metals are not suitable for fluorescence enhancement in the UV region as their plasmonic bands are respectively centered at ~400 nm and ~530 nm. However, metals of the boron group (Al, Ga, In, Tl) offer low absorption losses and strong plasmons centered at ~300 nm, making them good candidates for the design of plasmonic nanostructures for use in the UV. In this project, we are developing indium-based nanostructures capable of enhancing the native fluorescence of amino acids and proteins, biomolecules with a weak intrinsic fluorescence which are hindering their sensitive detection by fluorometric methods. Spherical core-shell indium@silica nanoparticles are synthesized using a hot-injection polyol method in order to obtain nanoparticles of approximately 80 nm with a plasmonic band centered at ~310 nm. Moreover, because the distance between the metallic core and the fluorophore must be optimised to avoid fluorescence quenching, a thin silica shell is grown on the indium core as a protective dielectric layer. Different fluorescence enhancement factors have been obtained by varying the spacer shell thickness. Finally, we have demonstrated the potential of indium in the design of bioprobes by measuring large fluorescence enhancement factors for two model fluorophores, Carbostyril 124 and tryptophan, one of the amino acids (with tyrosine and phenylalanine) responsible for the intrinsic fluorescence of proteins.

8957-22, Session 5

Metal-enhanced fluorescence of chlorophylls in light-harvesting complexes coupled to silver nanowires

Magdalena A. Twardowska, Dorota Kowalska, Maria Olejnik, Sebastian Mackowski, Nicolaus Copernicus Univ. (Poland)

Collective oscilation of free electrons in metalic nanoparticles, called localized surface plasmon resonance, can enhance the emission intensity of a fluorophore.[1]

In our experiment we examine the influence of plasmon excitations in silver nanowires (AgNWs), synthesized by the polyol method, on the fluorescence intensity and photostability of the peridinin-chlorophyll-protein (PCP) complex, isolated from algae. Fluorescence maps and kinetics were measured by wide-field microscope Nikon Ti-U equipped with iXon EMCCD detector. Irrespective of the sample preparation method, concentration of PCP complexes in the sample and excitation wavelength, we observed strong increase of the fluorescence intensity of the PCP complexes in the vicinity of the AgNWs. We also observed extremely bright hot-spots at the ends of the AgNWs.[2-3] Plasmon excitations in AgNWs do not affect photostability of PCP.[4]

Our results confirm that metallic nanoparticles can be applied for controlling the optical properties of biomolecules via plasmon excitations.

Financial support from the WELCOME program Hybrid nanostructures as a steppingstone towards efficient artifical photosynthesis awarded by the Foundation for Polish Science is gratefully acknowledged.

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8957-23, Session 5

Plasmonic hybrid nanostructure with controlled interaction strength

Justyna K. Grzelak, Bartosz Krajnik, Nicolaus Copernicus Univ. (Poland); Mark D. Thoreson, Purdue Univ. (United States); Piotr Nyga, Institute of Optoelectronics, Military Univ. of Technology (Poland); Vladimir M. Shalaev, Birck Nanotechnology Ctr., Purdue Univ. (United States); Sebastian Mackowski, Nicolaus Copernicus Univ. (Poland)

One of the major problems in design of plasmonic hybrid nanostructures is the ability to control the strength of the interaction[1]. While precise control of the distance has been recently demonstrated on a single molecule level, realizing similar scenario for highly concentrated sample still remains a challenge.

In this work we describe an approach of fabricating planar plasmonic structure with well-defined separation between metallic layer (silver island film) and light-harvesting complex, peridinin-chlorophyll-protein (PCP) of the dinoflagellate Amphidinium carterae.

The control is obtained by gradual variation of the dielectric spacer between this two nanostructures, its thickness increases from 0 to 46 nm over the length of the coverslide.

4-µl droplets of the PCP reconstituted with Chl a as well as a mixture with Chl b were deposited at several points across the substrate which corresponds to different values of the dielectric spacer.

First, in both cases we detect no changes of the shape of the fluorescence emission as a function of the spacer thickness. The emission intensity features the dependence similar to the one observed at a single molecule level.

These changes are accompanied with dramatic shortening of the fluorescence transient characteristic for thin spacers (<16 nm). We observe the strongest effect for excitation at 640 nm, which is resonant with the plasmon maximum of the silver island film.

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Financial support from the WELCOME program “Hybrid nanostructures as a stepping-stone towards efficient artificial photosynthesis” awarded by the Foundation for Polish Science is acknowledged.

8957-24, Session 5

Dependence of metal-enhanced fluorescence on surface roughness

Alexandre François, Beniamino Sciacca, Agnieszka Zuber, Elizaveta Klantsataya, Tanya M. Monro, The Univ. of Adelaide (Australia)

Metal Enhanced Fluorescence (MEF) takes advantages of the coupling between surface plasmons, in either a metallic thin film or metallic nanoparticles, and fluorophores located in proximity of the metal, yielding an increase of the fluorophore emission. While MEF has been widely studied on metallic nanoparticles with the emphasis on creating brighter fluorescent labels, planar surfaces have not benefitted from the same attention. Here we investigate the influence of the surface roughness of a thin metallic film on the fluorescence enhancement. 50nm thick silver films were deposited on glass slides using either thermal evaporation with different evaporation currents or an electroless plating method based on the Tollens reaction to vary the surface roughness. Multiple layers of positively and negatively charged polyelectrolytes were deposited on top of the metallic coating to map out the enhancement factor as function of the gap between the metallic coating and the fluorophore molecules covalently bound to the last polyelectrolyte layer. We show that fluorescence is enhanced by the presence of the metallic film, and in particular the enhancement factor increases from a factor 10 to 40 for roughness of 3 nm to 8 nm respectively. Although these enhancement factors are modest compared to the enhancement produced by complex metallic nanoparticles or nano-patterned metallic thin films, the thin films used here are capable of supporting a plasmonic wave and offer the possibility of combining different techniques, such as surface plasmon resonance (with its higher refractive index sensitivity compared to localised plasmons) and MEF within a single device.

8957-25, Session 5

Metal-enhanced fluorescence: effect of surface coating

Marjorie Lismont, Univ. of Liège (Belgium); Alexandre François, Institute for Photonics and Advanced Sensing (Australia); Laurent A. Dreesen, Univ. of Liège (Belgium); Tanya M. Monro, Institute for Photonics and Advanced Sensing (Australia)

Among the emerging treatments for cancer, Photodynamic Therapy (PDT) is thought to be one of the most promising. PDT uses light sensitive molecules, or photosensitizer, to produce, under specific irradiation, toxic reactive oxygen species (ROS) to kill cancer cells. However, the amount of ROS generated is limited by both the fluorescence lifetime of the photosensitizer and its concentration around the cancer cells. Metal Enhanced Fluorescence (MEF), a phenomenon arising when a fluorophore is in closed proximity to a metallic structure such as metallic films or nanostructures, is seen as a way to solve these problems by reducing the fluorescence lifetime and increasing the fluorescence emission of the fluorophore. Protoporphyrin IX (PpIX) is a commonly used photosensitizer to treat skin cancers, which presents an intense absorption band around 400 nm while emitting around 630 nm. Because silver nanoparticles (Ag NPs) exhibit a strong Localized Surface Plasmon Resonance (LSPR) around 400 nm, MEF of the PpIX is expected when immobilized onto Ag NPs.

Here, we investigate the relevant parameters influencing the coupling effects between the LSPR in Ag NPs and PpIX attached onto the Ag NPs surface when the Ag NPs are dispersed in solution or electrostatically bound to a glass slide. In particular, we study the distance-dependent of MEF by applying multiple layers of polyelectrolyte to progressively increase the distance between Ag NPs and PpIX, covalently bond to the last polyelectrolyte layer as well as exploring the use of Ag NPs of different sizes ranging from 40 to 80 nm.

8957-26, Session 6

Interactions between localized surface plasmons and molecular resonances

Gülis Zengin, Tina Gschneidtner, Tomasz J. Antosiewicz, Peter Johansson, Kasper Moth-Poulsen, Timur Shegai, Mikael Käll, Chalmers Univ. of Technology (Sweden)

Molecular plasmonics is the study of interactions between plasmonic nanostructures and molecules. It has been the basis for fundamental understanding of light-matter interactions and development of many technological applications, such as biological and chemical sensing, plasmon-enhanced spectroscopies, optical switches, and plasmon-enhanced dye sensitized solar cells. Organic chromophores interacting with plasmonic nanostructures constitutes an important part of the molecular plasmonics field. Rhodamine 6G is one of the organic chromophores that has been widely studied with the interaction of silver nanostructures especially in the context of single molecule surface-enhanced Raman spectroscopy. We observed spectral dips in the Rayleigh scattering of single silver nanoparticles covered with Rhodamine 6G. This was achieved by using a novel way of adsorbing Rhodamine 6G on silver surface via covalent thiol bonds. This new method made it possible to adsorb Rhodamine 6G densely on silver nanoparticles in the form of self-assembled monolayers. Mie theory calculations suggest that surface-enhanced absorption significantly contributes to these spectral dips. The strength of molecule-plasmon interactions is strongly affected by the properties of plasmonic nanoparticles and chromophores. We observed strong transparency dips in Rayleigh scattering when silver nanorods were covered with J-aggregates. By decreasing the radiative damping of plasmonic particles through reducing the particle size, and by using J-aggregates, which exhibit high oscillator strength and narrow transition linewidth, we show that it possible to approach the strong coupling regime.

8957-27, Session 6

Variation of the photothermal effect for cancer cell inactivation with localized surface plasmon resonance on Au nanorings of different geometries

Yi-Chou Tu, Che-Kuan Chu, Yu-Wei Chang, Hung-Yu Tseng, Yean-Woei Kiang, Chih-Chung Yang, National Taiwan Univ. (Taiwan)

Bio-conjugated Au nanoring (NRI) solutions with various NRI geometries

for inducing different localized surface plasmon (LSP) resonance wavelengths are fabricated based on an on-substrate fabrication and surface modification procedure. In this procedure, a few reactive ion etching and Au deposition processes are applied to form Au NRIs on substrate. After the bio-conjugation process, the Au NRIs are transferred into water. These Au NRI solutions are used for comparing their effectiveness of cancer cell inactivation through the photothermal effect. With three LSP resonance wavelengths at 1050, 1180, and 1270 nm of the Au NRI solutions and two excitation lasers at 1060 and 1315 nm in wavelength, the threshold laser intensities for the inactivation of HepG2 liver cancer cell under different Au NRI and excitation laser combinations are calibrated. It is found that the photothermal therapy is quite effective with the Au NRI of 1270 nm in LSP resonance wavelength when either the laser of 1315 or 1060 nm is used. With the Au NRI of 1050 nm in LSP resonance wavelength, only the laser of 1060 nm can lead to effective photothermal therapy. With the Au NRI of 1180 nm in LSP resonance wavelength, neither laser can result in effective photothermal therapy. Those differences in photothermal effect are attributed to the different absorption cross sections between those NRI samples. They are also related to the function of bio-conjugation. In other words, the efficiency of cell uptake is another crucial factor for the effectiveness of photothermal therapy.

8957-28, Session 6

Using hybrid modes in surface plasmon resonance bio-sensors for low-concentration target probing

Mitradeep Sarkar, Maha Chamtouri, Alexandra Sereda, Julien Moreau, Mondher Besbes, Anne-Lise Coutrot, Michael T. Canva, Lab. Charles Fabry, Institut d'Optique Graduate School (France)

Surface Plasmon Resonance (SPR) biosensors sensitive to trace concentrations of target bio-molecules are required for certain medical applications such as cancer bio-marker or PCR-free DNA hybridization detection. Most biochip systems based on surface plasmon excitation, have limited performance for such low concentration probing. Our aim is to surpass these limits by micro-nano-structuration of the biochip surface.

We have extensively studied periodic 2D metallic ribbon structures, with plasmon excitation parallel and perpendicular to the ribbons. We classify the modes in these structures either as Propagating Plasmons (PP) with symmetric lineshape or Coupled-Localized Plasmons (CLP) with asymmetric Fano lineshapes. Such CLP modes have also been studied in 3D structures such as nano-cylinders or nano-cubes. By manipulating these modes, we have optimized our structures to have field enhancement at specific locations. By selective localization of target molecules in these specific areas, enhancement of the sensor performance for low concentration probing is demonstrated.

Since we work with trace targets immobilized on a metal surface, the structures must have high sensitivity in vicinity of the metal surface and if possible be less sensitive to the changes in the bulk solution. In order to evaluate such sensors we introduce convenient parameters such as Sensitivity Enhancement Factor (SEF) and Sensitivity Figure of Merit (SFOM) which takes into account these features and also the concentration of target molecules required for the analysis.

We show by numerical simulations and experimental results that this can help to increase the limit of detection of bio-sensors in terms of target concentrations.

8957-29, Session 6

In-vivo gold nanorod imaging for brain tumor delineation

Kevin C. Seekell, Will Eldridge, Duke Univ. (United States); Christy Wilson, Gerald Grant, Stanford Univ. (United States); Adam Wax, Duke Univ. (United States)

Glioblastoma multiforme (GBM) is the most aggressive and deadly form of primary brain tumor. Treatment of GBM consists of surgical resection followed by radiation or chemotherapy. However, complete resection of the tumor is difficult due its visually indistinct margins. Surgeons therefore require a method of delineating brain tumor margins during the surgical procedure.

We propose the use of immunolabeled gold nanoparticles (NPs) as molecular contrast agents for brain tumor delineation. NP geometries such as gold nanorods and nanoshells may be tuned to have enhanced optical absorption in the tissue optical window (600-1200nm) where absorption from hemoglobin and water is minimal. Our previous studies have shown that gold nanorods conjugated with antibodies targeting Epidermal Growth Factor Receptor (EGFR) specifically bind onto EGFR(+) GBM tumors with minimal binding to the surrounding EGFR(-) normal tissues. Bound nanorods absorb the diffusely scattered light emitted by solid tissues.

In this study, a custom, portable darkfield fiber optic probe was designed to image gold nanoparticles bound to in-vivo brain tissues. High power LED sources provide an affordable illumination source for highlighting nanoparticle absorption. A novel GRIN lens configuration directs the illuminating light at an oblique angle so that only the scattered light from the tissue is collected and imaged. For in-vivo studies, GFP-expressing GBM cells are injected into the brains of nu/nu mice. After incubation with covalently conjugated anti-EGFR NPs, tissues are imaged using the darkfield probe. Regions with high NP density also exhibit high GFP fluorescence, indicating that immunolabeled NPs effectively label GBM tumors in-vivo.

8957-30, Session 7

Size and wavelength dependency of saturable and reverse saturable scattering by a single gold nanosphere embedded in dielectric material

Yen-Ta Huang, Hsueh-Yu Wu, Hsuan Lee, National Taiwan Univ. (Taiwan); Ryosuke Oketani, Yasuo Yonemaru, Osaka Univ. (Japan); Tung-Yu Su, National Taiwan Univ. (Taiwan); Masahito Yamanaka, Satoshi Kawata, Satoru Shoji, Katsumasa Fujita, Osaka Univ. (Japan); Shi-Wei Chu, National Taiwan Univ. (Taiwan) and Molecular Imaging Ctr., National Taiwan Univ. (Taiwan)

Gold nanoparticles are one of the most promising candidates in the application of optical nonlinearity due to their field enhancement caused by localized surface plasmon resonance (LSPR). Recently, we found, for the first time, saturable and reverse saturable scattering of single gold nanosphere (GNS) by continuous wave laser with wavelength close to LSPR. Combined with saturated excitation microscopy, which is developed by the authors, the nonlinear scattering property allows resolution better than 100-nm, providing an attractive superresolution contrast agent without bleaching. However, the mechanism of the exceptionally strong optical nonlinearity was still not fully clear. Borrowing idea from saturation of fluorescence, a tentative two-level model is proposed. According to the model, less intensity is required to reach saturation when excitation wavelength is closer to LSPR transition. In addition, the saturation intensity should be inversely proportional to the cross section. To test this assumption, we measured the saturation intensities with several different wavelengths (785, 592, 561, 532, 405 nm), and found that the saturation intensity did decrease as the wavelength approaching LSPR peak. With four different particle sizes (80, 40, 60, 20 nm), we confirmed that scattering from smaller GNSs are harder to saturate. Based on these results, the mechanism of nonlinear scattering can be connected to nonlinear absorption behavior of plasmonic materials. More quantitative results will be presented in the conference.

8957-31, Session 7

Three-dimensional light manipulation using plasmonic micro projector

Chia Min Chang, National Taiwan Univ. (Taiwan) and Academia Sinica (Taiwan); Ming Lun Tseng, National Taiwan Univ. (Taiwan); Bo Han Cheng, Academia Sinica (Taiwan); Cheng Hung Chu, You Zhe Ho, Hsin Wei Huang, Hung-Kuei Tsai, Kuang Sheng Chung, I-Da Chiang, Yueh-Hung Cheng, National Taiwan Univ. (Taiwan); Yung-Chiang Lan, National Cheng Kung Univ. (Taiwan); Ding-Wei Huang, National Taiwan Univ. (Taiwan); Ai Qun Liu, Nanyang Technological Univ. (Singapore); Din Ping Tsai, National Taiwan Univ. (Taiwan) and Academia Sinica (Taiwan)

Using nanostructures to manipulate surface plasmon polariton (SPP) plane waves is an important issue. The interactions of plasmonic nanostructure on SPP wave involve not only the in-plane behavior, but also out-of-plane scattering which is captured as the far-field radiation. Recently, three-dimensional focusing and diverging of SPP waves by a quarter circular structure composed of Au nanobumps were studied. The Au nanobumps confer additional three-dimensional propagating wave vectors on SPP wave for departing from surface. It is possible to manipulate the three-dimensional plasmonic scattering by arranging the Au nanobumps.

In this work, we manipulate the scattering of SPP waves by various plasmonic structures composed of arranged nanobumps on a gold thin film. Upon controlling the geometry of the plasmonic structures, the height, position, and pattern of scattered light can be modified as desired. It provides a simple and efficient way to project a specific light pattern into free space, and demonstrate the capability of three-dimensional light manipulation. By precisely designing a particular curved structure with appropriate radius of curvature and adjacent interspacing of nanobumps, we can construct a clear single focusing spot at a specific altitude. The irregular light patterns of the scattering of designed structures are observed at any observation plane, except for the scattering-light-focal plane where observing the focusing spot of curved structure. When the focal plane is shifted to this scattering-light-focal plane, the “NTU” light patterns are clearly observed. These results confirm the controllability of the focused spot in three-dimensional space by settling curved structures.

8957-32, Session 7

Manufacture and characterization of silver nanoparticles coated with silicon oxide to kill E. coli bacteria

Rodrigo E. Jones Estrada, Jesus I. Salomon Garcia, Instituto Politécnico Nacional (Mexico); Diogo B. Almeida, Univ. Estadual de Campinas (Brazil); Ernesto Jimenez Villar, Univ. Federal de Pernambuco (Brazil); Tupak E. García Fernández, Univ. Autónoma de la Ciudad de México (Mexico); Carlos Lenz Cesar, Univ. Estadual de Campinas (Brazil); Eugenio Rodríguez Gonzalez, Instituto Politécnico Nacional (Mexico)

Silver is traditionally known as a bactericidal agent, and silver salts (nitrate, lactate and silver sulfadiazine) are products widely used in bactericidal treatments. Recent studies have shown that antimicrobial formulations in the form of nanoparticles (NPs) of metal may be used as a bactericide effective. In the case of metal nanoparticles and silver specifically, it is known that if the frequency excited at the absorption of the nanoparticle, it produces the surface plasmon resonance and the surface charge cloud may move to the frequency of the external electric field producing intensifying the electric field on the surface of the nanoparticle. These surface effects can be exploited, they increase the bactericidal properties of compounds containing silver NPs. Due to the toxicity of silver in the human body, is proposed nanoparticles coated with a layer of silicon oxide.

In this paper we describe the fabrication of silver NPs for use as a bactericide. Silver nanoparticles were produced by the laser ablation technique in liquid medium. A power laser (20mJ / pulse) is focused on the surface of a silicon substrate submerged in water. With each pulse of the laser, a solid volume is ejected into the surrounding liquid, and rapidly condenses If small nanoparticles. AgNO3 subsequently added to the solution, forming Ag nanoparticles coated with a thin layer of SiO2

NPs containing these solutions are characterized by various techniques (UV-vis, DRX, HRTEM) to understand the mechanism of formation thereof and evaluate the feasibility of controlling the size of the NPs in terms of the growth parameters.

8957-33, Session 7

Dual-mode spectroscopy using plasmon waveguide resonance sensors for thin film investigation

Farshid Bahrami, Univ. of Toronto (Canada); Mathieu Maisonneuve, Michel Meunier, Ecole Polytechnique de Montréal (Canada); J. Stewart Aitchison, Mo Mojahedi, Univ. of Toronto (Canada)

A novel approach is proposed to decouple the surface and background effects in surface plasmon resonance biosensors. This method is based on plasmon waveguide resonance (PWR) sensor which can perform sensing with two different polarizations. The PWR sensor consists of a glass substrate, a thin metallic layer, and a dielectric layer on the top of the metal. The role of the metallic layer is to excite the dielectric waveguide modes (transverse magnetic (TM), and transverse electric (TE)) under certain conditions. The large probing depth of the TM polarized light in the PWR sensor makes it suitable for bulk sensing, while the small probing depth of the TE polarized light is ideal for surface sensing. The wavelength of operation and layer thicknesses are optimized with a genetic algorithm to acquire the best performance. The optimized sensor is then fabricated and the biotin-streptavidin system is used to investigate the sensing response. The phosphate buffered saline (PBS) with two different concentrations of streptavidin (1µg/ml and 10µg/ml) was passed over the biotinylated surface for surface sensitivity measurement and three different solutions with different refractive indices were flown for testing bulk sensitivity. The measured limit of detection for TE and TM polarizations are 9ng/ml and 55ng/ml respectively and the measured resolutions are 9.6?10-6 RIU and 2.5?10-6 RIU. Finally it is shown that by solving a system of linear equations, the biomolecular layer thickness and the background refractive index can get decoupled from the variations in resonance angles.

8957-34, Session 7

Physical mechanism of Au nanopore formation on pyramid using electron beam irradiation

Tokutaro Yamaguchi, Myoung Jin Park, Sun Moon Univ. (Korea, Republic of); NamKyou Park, Seoul National Univ. (Korea, Republic of); Seong Soo Choi, Sun Moon Univ. (Korea, Republic of)

We have fabricated the Au nanopores on the pyramidal oxide array using Si microfabriccation technology.

The 20 nm Au and 5 nm Cr was deposited on top of the oxide pyramid followed by FIB, FESEM electron beam. The solid state surface modiication model for FESEM wiill be presented.

Conference 8958: Bioinspired, Biointegrated, Bioengineered Photonic Devices II

BiOS SPIE Photonics West

Saturday - Sunday 1 –2 February 2014

Part of Proceedings of SPIE Vol. 8958 Bioinspired, Biointegrated, Bioengineered Photonic Devices II

8958-1, Session Key

Silk biomaterials: a versatile material platform for photonics *(Invited Paper)*

Fiorenzo G. Omenetto, Tufts Univ. (United States)

Biomaterials offer opportunities for devices that operate at the interface of the biological and technological worlds. Stringent requirements on material form and function are imposed when operating at the nanoscale in a photonic or optoelectronic context. In this talk we will present recent progress on the use of silk fibroin as the material base for micro- and nanostructured optical materials. Fabrication strategies and devices such as silk-based 2D and 3D photonic crystals, lasers, therapeutic mirrors, and biologically active photonics will be described as some examples of the possible applications that this water-processed, biocompatible material offers.

8958-2, Session 1

Bio-inspired photonic materials

Mathias Kolle, Massachusetts Institute of Technology (United States) and Harvard School of Engineering and Applied Sciences (United States); Joanna Aizenberg, Harvard School of Engineering and Applied Sciences (United States)

The precise control of light - matter interactions is crucial for the majority of known biological organisms in their struggle to survive. Many species have evolved unique methods to manipulate light in their environment using a variety of physical effects including pigment-induced spectrally selective absorption or light interference in photonic structures consisting of micro- and nano-periodic material morphologies. In their optical performance, many of the known biological photonic systems are subject to selection criteria not unlike the requirements faced in the development of novel optical technology. For this reason, biological light manipulation strategies provide inspiration for the creation of tunable, stimuli-responsive, adaptive material platforms that will contribute to the development of multifunctional surfaces and innovative optical technology. Here, we present the investigation of a bio-inspired elastically deformable photonic fiber material and elaborate on its unique tunable optical properties and potential for use in light guiding and strain or deformation sensing. The fibers that have a diameter of 60 – 200μm are manufactured by wrapping a bilayer of two optically distinct transparent elastomers around an elastic core fiber to form a cladding with up to 150 individual layers. The fibers' reflection can initially be tailored to any desired range in the UV, visible or IR by adjusting the thickness of the individual layers. After fiber formation, the reflection band can be tuned reversibly by applying a longitudinal or compressive strain making these fibers promising candidates for dynamic textiles or light-guiding and optical strain sensing applications in a biomedical context.

8958-3, Session 1

Self-formed ultraefficient resonators in mollusk shells

Seung Ho Choi, Young L. Kim, Purdue Univ. (United States)

Although irregular open nanostructures are typically inadequate for achieving strong light-matter interactions, incorporating irregularity can be advantageous as an alternative synthesis strategy, which is not affected by unavoidable structural variations and imperfections. We propose a framework to capitalize on natural disordered nanostructures as highly efficient optical resonators for light confinement and amplification. As one of the wondrous nanocomposite found in nature, the iridescent colors of mother-of-pearl (nacre) have been studied conventionally in terms of diffraction and interference. Surprisingly, we reveal that their main building block is centered on irregular and disordered nanostructures, in which disorder-driven resonances can be self-formed by multiple scattering without relying on well-configured closed cavities. We further demonstrate that the highly multilayered nanostructures of nacre can serve as a new class of disordered resonators to realize low lasing threshold and high energy conversion efficiency. Multiple resonances in such nanostructures, which are formed closely in frequency and space, can easily be overlapped to form hybridized states. This ensemble acting of multiple resonances drastically increases the effective cavity size, boosting light-matter interactions. For example, lasing action can be achieved using an edible food dye with a low quantum yield. Indeed, while ordered and closed resonators are commonly thought to be crucial, this biogenic approach can offer a novel strategy for designing and fabricating photonic nanostructures. The simplicity and efficiency of the natural resonators will open the new possibility of studying light propagation in complex media, measuring photoluminescence properties, and developing cost-effective photonic devices.

8958-4, Session 1

Biologically inspired microscope slides for neurochemical detection

Young-Jae Oh, Jae-Jun Kim, Ki-Hun Jeong, KAIST (Korea, Republic of)

Moth eyes in nature suppress the reflection by nanopillar arrays onto the air-cornea interface. The nanopillared surface provide low-refractive index layer with large surface area. Inspired from the moth eyes, we developed microscope slides with large-area glass nanopillar arrays for neurochemical detection. Thin silver film deposition (10 nm) onto glass wafer and thermal annealing constructed nanoislands as etch mask and following reactive ion etching defined large-area glass nanopillar arrays. Fill-factor and height of glass nanopillar arrays were controlled by silver masks and etching time. The height and fill-factor controlled glass nanopillar arrays provide autonomous antireflection in media with diverse refractive indices, which allow exceptionally high contrast imaging within aqueous environments in visible region. Moreover, metal deposition onto the glass nanopillar arrays offers nanogap-rich three-dimensional metal nanoislands for plasmonic biosensing. Nanogap distances between silver nanoislands were reduced by controlling thermal deposition thickness to provide high enhancement factor (> 10^7), therefore, high density nanoislands with small nanogaps enable highly intense surface-enhanced Raman spectroscopy (SERS). Combining the two novel functions, i.e., autonomous antireflection and nanogap-rich plasmonic structures was successfully achieved by double-side fabrication of the microscope slides. SERS signals were significantly improved by about 20 percent by antireflective glass nanopillar arrays, which enable the highly sensitive neurochemical detections. We believe that the simple, low-cost, and large area fabrication with outstanding advances can give great opportunities for advanced applications in a variety of biophotonic fields.

8958-5, Session 1

Toward bioinspired nanostructures for selective vapor sensing *(Invited Paper)*

Radislav A. Potyrailo, GE Global Research (United States)

Vapor sensors have their niche for vapor detection when an unobtrusive, low-power, and cost-sensitive technical solution is required. Unfortunately, existing vapor sensors often degrade their vapor-quantitation accuracy in the presence of high levels of interferences and cannot quantitate several components in complex gas mixtures. Thus, new sensing approaches are required with improved sensor selectivity. This technological task can be accomplished by the careful design of sensing materials with new performance properties and coupling these materials with the suitable physical transducers. In this talk, we will present our approach for selective vapor sensing by taking advantage of the hierarchical photonic nanostructure formed in the scales of Morpho butterfly wings. Upon interactions with different vapors and mixtures of vapors, such photonic structure produces remarkably diverse differential reflectance spectra. The response selectivity of iridescent scales of the Morpho butterfly wings dramatically outperforms existing nano-engineered photonic sensors.

8958-6, Session 2

Learning from nature: the retina as a discrete optical system *(Invited Paper)*

Jochen R. Guck, Technische Univ. Dresden (Germany) and Univ. of Cambridge (United Kingdom); Zuzanna Blaszczak, Univ. of Cambridge (United Kingdom); Moritz Kreysing, Ludwig-Maximilians-Univ. München (Germany) and Max-Planck-Institut für molekulare Zellbiologie und Genetik (Germany)

Evolution notoriously fumbled the vertebrate eye, which is inverted with respect to its optical function: light must pass through several tissue layers before reaching the light-sensitive photoreceptor cells on the back of the retina. While cells are mostly transparent, they are phase objects that differ in shape and refractive index. Any image that is projected through layers of cells will normally be distorted by refraction, reflection, and scattering. We have uncovered a number of ingenious adjustments that nature has implemented to optimize this apparently unfavorable situation. Müller (glial) cells span most of the retina, have a higher refractive index than the surrounding, and act like living optical fibers. Their combined parallel arrangement is reminiscent of fiber-optic plates used for low-distortion image transfer. Underneath, the nuclei of the photoreceptor cells in nocturnal mammals display a unique inversion of their chromatin distribution to serve as micron-sized ball lenses. Direct experiments, analytical models and FDTD simulations suggest that the arrangement of these nuclei in columns greatly improves transmission characteristics by a reduction of scattering and an effective channeling of light through the outer nuclear layer. Recently, we have described the functioning of the so-called grouped retina in certain fish, which utilize a parabolic cup lined with a 1D photonic crystal to improve vision in their turbid habitat. Together these results change our understanding of the retina as an optical system, could stimulate and benefit from research on artificial discrete optical systems, and maybe even lead to novel optical biomimetics.

8958-7, Session 2

An ultrawide-angle artificial reflecting superposition compound eye

Chi-Chieh Huang, Hewei Liu, Univ. of Wisconsin-Madison (United States); John A. Rogers, Univ. of Illinois at Urbana-Champaign (United States); Hongrui Jiang, Univ. of Wisconsin-Madison (United States)

In nature, reflecting superposition compound eyes (RSCEs) found in lobsters and other crustaceans are extraordinary imaging systems with numerous optical features such as wide-angle field of view (FOV), minimum chromatic aberration, great sensitivity to light and high acuity to movement. Here, we present life-sized, ultra-wide-angle artificial RSCEs as optical imaging devices inspired by the unique designs of their natural counterparts. The configuration of artificial RSCEs consists of an array of mirrored, high-aspect-ratio micro-square-tubes array omni-directionally arranged onto a thin, transparent, flexible hemispherical membrane with radius of curvature of 1.2 cm. The height, opening, and inter-tube spacing of each micro-square-tube were 60, 30 and 15 ?m, respectively. Such devices, fabricated by a peeling micro-transfer printing method, can form real, erect images based on reflection and hence avoid chromatic aberration and low transmittance due to dispersion and absorption by the device materials, respectively. Our results demonstrate an exceptional FOV up to 165 degrees without distortion, minimum chromatic aberration, and imaging performance comparable to that of a conventional refractive lens without any post-image processing operations. Together with an augmenting cruciform pattern surrounding each focused image, we have realized a bio-inspired, ultra-wide-angle artificial RSCE capable of real-time motion-tracking and fine imaging at the visible wavelengths. In a general sense, our work can be a significant inspiration for advancing the development of a broad range of existing technologies used in military, security, medical imaging and astronomy.

8958-8, Session 2

Spectral and frequency-domain collagen detection of spherical ocular tissue

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Collagen is the main load-bearing component within the eye tissue, and the mechanical properties of these tissue depend on the collagen composition and organization. In the structure of eyeballs, especially the sclera, collagen plays an important role in the maintaining form and function. To clarify these structural roles, many investigations were devised to quantify the patterns of collagen fiber arrangement, although most utilized invasive, destructive ways. In view of the shortcomings of these existing techniques, we recently developed a 3D optical scanner coupled to a collagen fiber optic probe to map the ocular sphere non-invasively. The fiber optic delivers a 365 nm excitation that is sine-modulated at 90 MHz. We use a cooled mini spectrometer in conjunction to a 380 nm high pass filter to monitor the spectral signals of the tissue. For the lifetime, we use the frequency domain method to determine the lifetime contributions of the tissue. This works by detecting the phase delay of the fluorescent emission with respect to the excitation light when the intensity of the excitation light is modulated. Both spectral and lifetime detections can be obtained simultaneously using a 60/40 beam splitter. These measures are taken at spots that are scanned across the ocular sphere using a stepper motor automated 3D scanner, at 7.2? steps yielding a potential grid of 25 latitude lines and 50 longitudinal lines. Using the combined 3D and dual spectral/lifetime optical detection, the cattle eyes can be monitored during a variety of stress and structure analysis.

8958-9, Session 2

Bio-inspired hemispherical compound eye camera *(Invited Paper)*

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Compound eyes in arthropods demonstrate distinct imaging characteristics from human eyes, with wide angle field of view, low aberrations, high acuity to motion and infinite depth of field. Artificial imaging systems with similar geometries and properties are of great interest for many applications. However, the challenges in building such systems with hemispherical, compound apposition layouts cannot be met through established planar sensor technologies and conventional optics. We present our recent progress in combining optics, materials, mechanics and integration schemes to build fully functional artificial compound eye cameras. Nearly full hemispherical shapes (about 160 degrees) with densely packed artificial ommatidia were realized. The number of ommatidia (180) is comparable to those of the eyes of fire ants and bark beetles. The devices combine elastomeric compound optical elements with deformable arrays of thin silicon photodetectors, which were fabricated in the planar geometries and then integrated and elastically transformed to hemispherical shapes. Imaging results and quantitative ray-tracing-based simulations illustrate key features of operation. These general strategies seem to be applicable to other compound eye devices, such as those inspired by moths and lacewings (refracting superposition eyes), lobster and shrimp (reflecting superposition eyes), and houseflies (neural superposition eyes).

8958-10, Session 3

Micovascular integration into porous polyHEMA scaffold

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Biomaterials elicit foreign body responses when implanted into living tissue. While biocompatibility has been improved, the fundamental aspects of tissue responses to biomaterials and their in vivo evaluation remain poorly appreciated. Here, we quantified microvascular growth into porous poly2-hydroxyethylmethacrylate (polyHEMA) with 40 and 80 µm nominal pore sizes at various timepoints after implantation in rat subcutis. Solid polyHEMA, silicone, and cotton materials were also studied for comparison. We observed angiogenesis into the material one week after implantation for both 40 and 80 µm porous polyHEMA. The rate of vascularization was greater in 80 µm polyHEMA with higher microvessel densities near the material and adjacent tissues one week and one month post-implantation. The microvascular density inside 80 µm polyHEMA reached a maximum one month after implantation. In 40 µm polyHEMA, the microvascular density increased gradually over the two months following implantation. After two months, vascularization in the material was similar for both 40 and 80 µm polyHEMA. Notably, despite similar levels of vascularization inside both porous materials at two months, the 80 µm polyHEMA elicited greater vascularization in the critical 100 µm margin of tissue closest to the implant compared to other materials. In all materials except 80 µm polyHEMA, we observed a narrow relatively avascular margin near the implant-tissue interface. We conclude that implanted 80 µm pore polyHEMA encourages vascularization, underscoring the importance of rigorous evaluation metrics to assess long-term performance of implanted biomaterials and guide future biomaterial optimization.

Supported in part by DARPA grant numbers W911NF-11-1-0119 and HR0011-13-2-0003, by the Fitzpatrick Institute of Photonics and by the Robert Jones Fund, Duke University. The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

8958-11, Session 3

Implantable bioabsorbable waveguide based oximetry

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Reconstructive surgical procedures are frequently performed to fix tissue deficiencies due to trauma, burns, or surgical removal. Assessment of the tissue blood flow and oxygenation and oxidative metabolism is critical for diagnosis of diseases and therapy after surgery [1]. For this purpose visible and near-infrared light signals are used on the target tissue and the change in the signal levels due to the light absorption by endogenous and exogenous chromophores are investigated to understand the condition of patients [2]. However, in-situ continuous monitoring and stimulation of the reconstructed tissues is currently not possible due to the lack of non-invasive technologies. For this purpose we developed implantable and bioabsorbable waveguides that can be used to sense the tissue oxygen change in targeted organs and tissues. We have designed, fabricated and characterized optical waveguides made of polylactic acid, poly(lactic-co-glycolic acid) and polyvinylpyrrolidone and we have used the waveguides to sense the oxygen change.

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8958-12, Session 3

Azobenzene based polymers as photoactive supports for cell growth applications

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Azo-polymers have been investigated for the large structural modifications this class of materials can undergo under light excitation. Cooperative photo induced isomerizations of the azobenzene molecular units can provide forces able to affect self-assembling processes in the liquid state or to lead to an efficient mass transport in the solid state resulting in large deformations of film surfaces with potential for a wide range of applications.

We will present studies performed on a class of azopolymers based on a polysiloxane matrix bearing specific chemical functions and which composition can be finely tuned for applications in the biological field. Depending on the chemical structure of the materials, systems able to produce photosensitive micellar architectures in aqueous solvents or photoactive surfaces with adjustable topographic properties have been produced. In the latter case the ability to control the surface shape at the optical wavelength scale is aimed at providing photoactive cell growth supports with tuneable properties for the investigation of the environment influence on the biological cell development. The topographic modifications being based on a reversible mass transport within the film, the surface chemical composition influence on the cell behaviour can be investigated independently. The optical properties of the materials will be presented and first studies showing the potential of the materials to modify the cell response to the surface will be presented. The stability of the films surface exposed to biological media and implications on the cell behaviour will also be addressed.

8958-13, Session 3

Smart transdermal vaccine delivery systems using intravital microscopic imaging

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Vaccines are one of the most effective ways to protect human health from various vaccine-preventable diseases, ranging from hepatitis and tuberculosis to seasonal flu. A variety of vaccine delivery systems have been investigated for facile and efficient immunization with patients' compliance[1]. Among them, transdermal vaccine delivery might be a strong alternative candidate due to well-designed immune systems in the skin tissues containing Langerhans and dendritic cells[2,3]. Intravital microscopy is used to observe real-time in vivo biological phenomenon at high resolution [4]. In this work, we carried out synthesis and imaging of hyaluronic acid[5] - ovalbumin (HA-OVA) conjugates as model vaccine system for developing smart transdermal vaccine delivery systems. HA-OVA conjugates were synthesized by site-specific reaction between HA-aldehyde and N-terminal amine group of OVA. The successful synthesis of HA-OVA conjugate was confirmed by 1H NMR, GPC and ELISA. Two-photon microscopy clearly visualized the effective transdermal delivery of HA-OVA conjugates labeled with Texas red. Remarkably, the transdermal delivery of HA-OVA conjugate via the skin of MHC-II+GFP+ mice resulted in statistically significant immunization according to the ELISA for the concentration of anti-OVA IgG in cardiac blood samples. Taken together, we could confirm the feasibility of HA-based transdermal vaccine delivery systems for further development[6].

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8958-14, Session 3

Shaping light for biomedicine *(Invited Paper)*

Kishan Dholakia, Univ. of St. Andrews (United Kingdom)

The shaping of light in amplitude, phase and polarisation is of utmost importance for the next generation of biomedical studies. Here I will describe our work on cell transfection, Raman and disordered materials including studies that are inspired by the biological world

In the area of cell transfection, i will show work using superresolved fields for transfection, transfection of stem cells and primary neurons. In the context of disorder, I will describe how we may improve penetration and explore new routes for spectral analysis of tissue

8958-15, Session 4

Optofluidic bioenergy: photobioreactors for solar energy conversion and to probe photosynthetic function *(Invited Paper)*

David D. Sinton, Univ. of Toronto (Canada)

Photosynthesis provides an elegant model for solar energy utilization and the conversion of CO2 into useful high energy products. Engineering practical photobioreactors to harness the photosynthetic process, however, is an on-going challenge. In this paper, I will overview our work on integrating optics and fluidics to achieve high-density photobioreactors. This work targets improvements in photobioreactor engineering as well as a new tools with which to probe the fundamentals of photosynthetic energy conversion.

I will introduce an optofluidic photobioreactor concept where photosynthetic bacteria are provided light and fluids via waveguiding structures. We demonstrate successful cultivation of biofilms in response to evanescent light generated through total internal reflection and also through surface plasmon resonance. A layered photobioreactor structure is presented whereby distinct layers employ plasmonic scatters to harvest portions of the spectrum – an approach inspired by the spectral and depthwise stratification of microalgae in natural environments. Plasmonic cultivation presents the additional opportunity to use the metal surface not only to deliver light, but also to capture electrons from the photosynthetic process. Direct electricity production from a plasmonically-excited biofilm is demonstrated with peak power of ~ 10 microW/m^2. Lastly I will describe our group's efforts in developing new analytical tools with which to understand photosynthetic microorganisms and increase their productivity. These efforts include densely multiplexed photobioreactors as well as single photosystem excitation via nanoresonators.

8958-16, Session 4

Biologically inspired asymmetric micro/nanostructures from firefly light organ for highly efficient light extraction

Jae-Jun Kim, Dongmin Keum, Ki-Hun Jeong, KAIST (Korea, Republic of)

Biological inspirations from natural photonic structures such as compound eyes or butterfly's wings have been intensively studied for novel imaging or display applications. Not only compound eyes or wing scales, bioluminescent organs are also expected that the organs efficiently manipulate the bioluminescent light in a simple manner and they can provide inspiration for illumination applications. In particular, a firefly light organ is a distinctive organ for delivering optical signals in sexual communication. Highly efficient emission of bioluminescent light is the first order basic requirement for successful communication for sexual selection. However, the cuticular structures except the light producing reactions have not been investigated. Here we report the biologically inspired asymmetric micro/nanostructures found in a firefly light organ, which exists on the abdominal segments of a firefly (Pyrocoelia rufa) in male. The physical dimensions were statistically extracted from the SEM images. The dimension of microstructures is about 10 ?m and the nanostructures are highly ordered. The angular transmittance of bioluminescent light (?= 560 nm) through the light organ cuticle was calculated by using a finite difference time domain (FDTD) method. The structures on a cuticle surface contribute to improve light extraction by the reduction of total internal reflection, compared with a smooth surface. The asymmetric micro/nanostructures were successfully fabricated by using two-step photolithography, surface energy modulation, and thermal reflow. Surface energy of the substrate was controlled to profile of photoresist after thermal reflow process. This device offers an effective illumination system for diverse lighting applications.

8958-17, Session 4

Understanding the Nanophotonic Light-trapping Structure of Diatom Frustule for Enhanced Solar Energy Conversion: A Theoretical And Experimental Study

Xiangfan Chen, Chen Wang, Evan C. Baker, Cheng Sun, Northwestern Univ. (United States); Cheng Sun, Dept. of Mechanical Engineering, Northwestern University (United States)

The recent design in nanophotonic light-trapping technologies offer promising potential to develop high-efficiency thin-film solar cell at dramatically reduced cost. However, the lack of cost effective scalable nanomanufacturing technique remains the main road-block. In nature, diatoms exhibit high solar energy harvesting efficiency due to their frustules (i.e., hard porous cell wall made of silica) possessing remarkable hierarchical nano-features optimized for the photosynthetic process through millions of years evolution. To explore this unique light trapping effect, different species of diatoms (Navicula and Coscinodiscus sp.) are cultured and characterized by Scanning electron microscope (SEM) and focused ion beam (FIB). Rigorous Coupled Wave Analysis (RCWA) and Finite-difference time-domain (FDTD) method are employed to numerically simulate the scattering effect. The absorption efficiency is significantly enhanced around 400nm and 700nm when the maximum electric field intensity overlaps the active layer. The transmission and reflection spectra are also measured by optical spectroscopy and the experimental results are in good agreement with numerical simulations.

The possibility of integrating controlled patterns and layers of diatoms into dye-sensitized solar cells (DSC) is also being explored. Using a LCD dynamic mask for full digital control of color and spatial patterns of the projected optical field in real time, we utilize a programmable light array projection system to quantitatively evaluate the kinetic characteristics and decipher the underlying mechanisms governing the migration of diatoms. The goal is to use this system as a manufacturing tool to guide the self-assembly of diatoms into a large, evenly dispersed and properly aligned arrays.

8958-18, Session 5

Smartphone-based molecular and chemical diagnostics *(Invited Paper)*

David Erickson, Cornell Univ. (United States)

Advancements in nanotechnology and microfluidics have enabled the development of lab-on-chip devices that can detect and quantify protein, genetic, and other biochemical markers of diseases with unprecedented precision. Unfortunately, despite years of promise, very few of these technologies have actually made inroads to the personalized healthcare market. A major reason for this is the majority of conditions most consumers wish to get tested for require only occasional monitoring and so are not compatible with a classic razor-and-blades deployment model. The result is that available personalized diagnostic devices are limited to conditions that require either frequent monitoring (e.g. glucose for diabetics) or "binary" results (e.g. pregnancy). In this talk I will first demonstrate that this roadblock to the deployment of lab-on-chip technology can be fundamentally altered by taking advantage of the now ubiquitous installed base of smartphone technology, and second show that that the fusion of physical sensing and molecular assays on mobile platforms will enable healthcare diagnostics that are far more telling than what is possible with either technology alone. Specific applications that will be discussed include: diet alkalinity monitoring, diagnosis of Kaposi's Sarcoma in limited resource settings, and personalized micronutrient monitoring.

8958-19, Session 5

Optical EEG (OEEG): a novel technique toward plug-and-play noninvasive brain imaging and human-machine interfacing

Ehsan Kamrani, Harvard Medical School (United States) and Polystim Neurotech. Lab., Polytechnique Montréal (Canada); Seok Hyun A. Yun, Harvard Medical School (United States)

The human brain dynamics can be studied based on the fast-neuronal and slow-hemodynamic signals. Referring to the available brain imaging techniques, the electroencephalography (EEG) and Magnetoencephalography (MEG) can only measure the fast-neuronal signal. Positron-emission-tomography (PET) and Functional-magnetic-resonance-imaging (fMRI) also can measure only the slow-hemodynamic signal. However, the functional-near-infrared spectroscopy (fNIRS) is the only imaging system capable of detecting both fast-neuronal and slow-hemodynamic signals.

In this research we have first implemented a miniaturized low-power fNIRS front-end using standard CMOS technology for monitoring the hemodynamic signals (HbO & HbR) in continuous wave (CW) and time-correlated single photon counting (TCSPC) modes of operation. A new on-chip implemented system (called Optical EEG: OEEG) is also proposed to estimate the neural activities based on the acquired optical data using fNIRS. This is the first proposed wholly optical system capable of monitoring neuronal and hemodynamic signals and it is the first step towards introducing an optical alternative system for EEG. In order to develop a real-time system, we have proposed detecting the stable, controllable and observable hemodynamic states of the brain, based on the continuous-discrete extended-Kalman-filter and bifurcation analysis of the balloon-model. It extracts and validates the states and verifies their stability, controllability and observability. The stable, controllable and observable states are used for further processing. In contrast to the other available complex techniques, the complexity of the proposed

Algorithm is moderate and its implementation requires only raw data) extracted directly from a continuous-wave fNIRS-channel. The results confirm the efficiency of the proposed technique in dealing with noise and movement-artifacts. It increases the signal-to-noise-ratio (SNR) and the speed.

8958-20, Session 5

Optofluidic bio-lasers: bridging photonics, nanotechnology, and biology *(Invited Paper)*

Xudong Fan, Univ. of Michigan (United States)

The optofluidic laser is a rapidly growing area that integrates microfluidics, miniaturized laser cavity, and laser gain medium in liquid. It is uniquely positioned to interface photonics, nanotechnology, and biology, thus having significant potential in both forefronts in novel photonic devices and bio/chemical sensing. Here I describe and envision a new type of optofluidic laser - optofluidic biolaser, that takes advantage of self-recognition, self-assembly, self-modulation, and energy harvesting capabilities of biomolecules. I will review its current implementations and discuss its future applications.

8958-21, Session PSun

When Birnam Wood comes to Dunsinane: copying nature in medical research

Bradley S. Tice, Advanced Human Design (United States)

The examination of biological structures that can be researched to design new functional models for ‘smart’ materials that can be used for medical purposes. The presentation will focus on the properties of a tree branch and the examination of the design of an ancient mechanical prosthetic limb, a toe, that has a ‘functional’ element that is lacking in todays designs of similar prosthetic devices.

8958-22, Session PSun

Algorithmic complexity and plant genetics

Bradley S. Tice, Advanced Human Design (United States)

The examination of plant DNA compression using an algorithm designed from algorithmic complexity. The algorithm is designed to compress sequential strings of DNA that are both random and non random. The algorithm is for use in agricultural and biological genetics.